Studies on smoking in patients with inflammatory bowel disease and liver transplant recipients

Frans van der Heide

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Contents

Chapter 1

Introduction and outline of the thesis

Introduction

Two important groups of patients for the gastroenterologist and hepatologist are patients with inflammatory bowel diseases (IBDs) and liver diseases. The two most common IBDs are Crohn's disease (CD) and ulcerative colitis (UC), and they are characterized by relapsing inflammation of the gastrointestinal tract. In CD the entire gastrointestinal tract can be involved, while in UC the inflammation is limited to the colon. The prevalence in Europe and North America of CD is about 50 to 200/100,000 and of UC about 120 to 200/100,000.[1] Patients with IBD may have a complicated disease course with a high need for immunosuppressants and surgery; CD patients with small bowel disease have a 70-80% chance of requiring surgery and 20-30% of UC patients need a colectomy.[1] The cause of IBD is thought to originate from a dysregulated immune system, but exact mechanisms are largely unknown. This dysregulated immune system is probably influenced by environmental factors and the genetic background. The last decade a still increasing number of genetic variants have been found to be associated with IBD. However, studies investigating interaction between these genetic variants and environmental factors are scarce.

The other important group is patients with liver diseases. The most prevalent liver diseases in Europe and North America are non-alcoholic fatty liver diseases, alcoholic liver diseases, viral hepatitis (Hepatitis B and C), primary sclerosing cholangitis, primary biliary cirrhosis and autoimmune hepatitis. All these liver diseases can lead to cirrhosis. Orthotopic liver transplantation (OLT) is an accepted therapy for end-stage liver disease. The prognosis of recipients has increased over the last decades with a 1-year survival rate of 85% and a 5-year survival rate of 75%. The most important causes of morbidity and mortality after OLT are malignancies and cardiovascular diseases. Therefore, one of the main goals of the follow-up of recipients after OLT should be prevention and early detection of malignancies and cardiovascular diseases. Smoking is a notorious risk factor for several malignancies and cardiovascular diseases, and therefore knowledge about the prevalence and effects of smoking in recipients of liver transplants is of utmost importance. However, studies about the role of smoking in recipients of liver transplants are scarce.

Besides the well-known effects of smoking on several malignancies, cardiovascular diseases and lung disease, smoking also affects the digestive organs. Especially the role of smoking in IBD is remarkable. Smoking has opposite effects on CD and UC; smoking seems detrimental for CD, but beneficial for UC. In this introduction, we describe this remarkable, opposite role of smoking between CD and UC; in section 1.2 the detrimental effects of smoking on CD, in section 1.3 the beneficial effects of smoking on UC and in section 1.4 some possible biological mechanisms involved in the effects of smoking in CD and UC. In addition, in section 1.5 we discuss smoking behaviour in OLT recipients and the effects of smoking after OLT.

Smoking and Crohn's disease

CD patients are more likely to be smokers than their matched controls, making smoking a risk factor for developing CD.[2-5] In a meta-analysis of 1,679 CD patients, active smokers had an odds ratio (OR) of 1.76 (95% confidence interval (CI): 1.40-2.22) for developing CD.[5]

Not only active smoking, but also passive smoking may be a risk factor for developing CD. However, information about passive smoking as a risk factor for developing CD is scarce and inconclusive. Some studies showed that CD patients were more often exposed to tobacco smoke in childhood than controls.[6,7] However, a meta-analysis showed no effect of passive smoking in childhood on CD, although a sub analysis based on effects of maternal smoking showed a positive association with CD (OR 1.3 (95% CI: 1.1–1.6)).[8]

In addition to the detrimental effect of smoking on the development, many studies showed that smoking was also unfavourable for the disease course of CD. Smoking CD patients had a higher need for hospitalizations,[9] steroids,[10-12] azathioprine [9-11,13] and surgery,[9,14-16] and had earlier and/or more often recurrence after surgery than non-smokers.[15,17-23] However, this detrimental effect of smoking on the course of CD was not confirmed by other studies.[24-34] In contrast to the detrimental effect of smoking, two recent studies showed that smoking protected against relapse after azathioprine withdrawal.[35,36] The literature on the effects of smoking is not only ambiguous, the effects of smoking also seem to depend on gender,[10,17,20,37] and disease location and severity.[18,38] The literature on the effect of smoking on the results of treatment with the anti-TNF-α antibody infliximab is also ambiguous. In a recent review two out of ten studies showed a higher response in non-smokers than in smokers, but the other eight studies showed no effect of smoking.[39] In a study comparing a top-down and a step-up strategy for the medical treatment of CD, smoking had no effect on the outcome either, including that of infliximab.[40]

Summarizing, active and maybe also passive smoking are risk factors for developing CD. Smoking may also be detrimental for the disease course of CD and may affect response on therapy, but literature is not conclusive on these matters.

Smoking and ulcerative colitis

Opposite to CD, UC patients are less likely to be smokers than controls, suggesting that smoking protects against developing UC. [3-5,41,42] In a meta-analysis of 2,459 UC patients active smokers had a decreased risk for developing UC (OR 0.58 (95% CI: 0.45-0.75)), and former smokers had an increased risk (OR 1.79 (95% CI: 1.37-2.34)).[5] The role of passive smoking for developing UC has also been studied. A protective effect of passive smoking in childhood was suggested,[43] but this was not confirmed in a meta-analysis.[8]

Smoking may also be beneficial for the disease course of UC. Smokers had a lower need for hospitalizations,[29,44] steroids [45] and a colectomy [16,26,45,46] than non-smokers. However, the positive effect of smoking on the colectomy rate was not always apparent in other cohorts.[25,27,44,47-49] Smoking cessation had detrimental effects on the course of UC, since patients who stopped smoking had a higher need for hospitalizations, steroids and azathioprine in the first year after stopping compared to continuing smokers.[50] Finally, smoking protects against primary sclerosing cholangitis,[51-53] an infamous extraintestinal disorder particularly associated with UC.

Summarizing, active smoking is protective and smoking cessation is a risk factor for developing UC. Smoking may also be detrimental for the disease course of UC, but as in CD, literature is not conclusive on this matter.

Pathophysiology of smoking in IBD

The cause of IBD is largely unknown, but it is thought to originate from a dysregulated immune system influenced by genetics, environmental factors and commensal intestinal bacteria.[54-56] It is not surprising that it is also unknown why smoking is detrimental for CD and beneficial for UC. Mechanisms that have been suggested to play a role are the Toll-like receptor-4-dependent pathway in macrophages,[57] the heme-oxygenase-1 pathway (HO-1) [58] and thrombogenic effects of tobacco on the intestinal microvasculature.[59,60] HO-1 is the rate-limiting enzyme involved in the breakdown of heme, yielding the end-products biliverdin, Fe^{2+} and carbon monoxide. Heme causes oxidative stress, while all three end-products have anti-oxidative, anti-apoptotic and anti-inflammatory properties. Induction of HO-1 was beneficial for the intestines in several animal models of oxidative injury and inflammation.

The understanding of the mechanism is complicated by the large number of components in cigarette smoke, of which nicotine and carbon monoxide are the most widely studied. Studies with nicotine showed that the detrimental effects of cigarette smoking on CD may be mediated by the binding of nicotine to the nicotinic acetylcholine receptor (nAChR) $\alpha 7$, which is an essential regulator of inflammation.[61,62] In TNBS-induced colitis in rats the colon tissue was damaged more seriously when the nAChR-$\alpha 7$ expression was up-regulated by exposure to cigarette smoke.[63] Another detrimental effect of nicotine was that it suppressed the clearance of bacteria by macrophages.[64] Clues for an association between UC and nicotine are the presence of nAChRs in colonic epithelium,[65] and that nAChR-$\alpha 5$ knockout mice had a more severe experimental colitis than wild-type mice.[66] Nicotine also has beneficial effects on epithelial mucus synthesis and gut motility, and it reduces the levels of pro-inflammatory cytokines and mucosal eicosanoids.[46,67-69] Finally, several intervention studies suggested a beneficial effect of nicotine in UC patients with active disease.[70,71] However, nicotine was not able to maintain remission of UC.[72] Surprisingly, nicotine enemas were also beneficial for CD patients with colitis.[73]

The second widely studied component of cigarette smoke is carbon monoxide. In CD, carbon monoxide may amplify the impairment in vasodilation capacity of chronically inflamed micro-vessels, resulting in ischemia, and perpetuation of ulceration and fibrosis.[74] Beneficial effects of carbon monoxide are inhibition of the lipopolysaccharide-induced expression of pro-inflammatory cytokines (TNF-α, IL-1β and macrophage inflammatory protein-1β) and increase of the anti-inflammatory cytokine IL-10.[75] Several interventions studies in mice showed beneficial effects of carbon monoxide; it ameliorated chronic intestinal inflammation in interleukin-10-deficient mice,[76] and it inhibited the intestinal inflammation in a TNBS-induced and DSS-induced colitis in mice with a decrease of TNF-a production and neutrophil infiltration into the intestinal mucosa.[77,78]

Summarizing, it is still unknown which exact mechanisms of several suggested mechanisms in IBD are involved in mediating the effects of smoking, and which component(s) of cigarette smoke is/are involved. Nicotine and carbon monoxide are the most studied components, and both have detrimental as well as beneficial effects on intestinal inflammation.

Smoking and liver transplantation

Only two studies, both from the United States, have studied the smoking behaviour in OLT recipients. DiMartini *et al*. prospectively studied the smoking behaviour in 33 patients transplanted for alcoholic liver disease. They found that more than 40% of patients with alcoholic liver disease were smoking after OLT, and that recipients resume smoking already at 3 months post-OLT and increase consumption of cigarettes over time.[79] Ehlers *et al*. retrospectively studied the smoking behaviour in 202 recipients before and after OLT. They reported a lifetime history of smoking before OLT of 60% and an active smoker rate of 15% after OLT. Twenty percent of smokers who quitted before OLT relapsed after OLT.[80]

The effects of smoking on the long-term course after liver transplantation have been studied more often. Smokers have an increased risk for malignancies after OLT,[81-83] especially recipients with alcoholic liver disease.[84-86] Smoking OLT-recipients also had a higher incidence of cardiovascular events,[87] and an increased risk of cardiovascular death and sepsis-related mortality compared with non-smokers.[88] In a study on 263 OLT recipients smokers had a higher risk for developing vascular complications, especially arterial complications after liver transplantation.[89] Finally, active smokers had a 92% higher rate of biliary complication rates compared with lifetime non-smokers in a study on 409 recipients.[90]

Summarizing, smoking is a risk factor for several complications and serious events after OLT, including for the most important causes of morbidity and mortality in these patient (malignancies and cardiovascular diseases), but literature on smoking behaviour in recipients is disappointingly scarce.

Outline of the thesis

The general aim of this thesis is to explore the role of smoking in the inflammatory bowel diseases Crohn's disease and ulcerative colitis, and the role of smoking in liver transplant recipients.

In chapter 2 we studied the smoking behaviour in patients with Crohn's disease and ulcerative colitis. For this purpose we used a written questionnaire about active and passive exposure to cigarette smoke, and about cessation plans of active smokers. The aim was to identify possible differences in smoking behaviour between CD and UC patients.

In chapter 3 and 4 the effects of active and passive smoking on the disease courses of CD and UC are studied. In chapter 3, we studied IBD patients from a university hospital and in chapter 4 IBD patients from a general hospital. Smoking behaviour was defined by using the same questionnaire as in chapter 2. Data on the disease course was obtained through retrospective analysis of the patient records.

Chapter 5 reports on the interaction between smoking and genetic background in CD. It is likely that the development of CD is partly caused by an interaction between the several identified genetic variants and smoking, but studies investigating this interaction are scarce. In this study we aimed to explore whether there are differences in CD associated genetic variants between CD patients stratified for active smoking at diagnosis and for passive smoking in childhood.

Chapter 6 is a laboratory study on the effects of smoking on the expression of HO-1.

The HO-1 pathway could be one of the pathways involved in the beneficial effects of smoking on UC. Induction of HO-1 was beneficial in several animal models of intestinal injury. Cigarette smoke is able to induce HO-1 in several human cells, but the effect of smoking on colonic HO-1 is unknown. Induction of colonic HO-1 by smoking could be beneficial for UC patients by protecting against development of UC and/or by ameliorating inflammation in established UC. We hypothesized that smoking increases the colonic HO-1 expression. For this purpose, we studied the effects of smoking on colonic HO-1 expression *in vitro*, in animals and in humans.

In chapter 7 we report about the smoking behaviour of liver transplant recipients before and after transplantation, and about the association of smoking with malignancies and cardiovascular diseases after transplantation. One of the aims was to define groups at risk for resuming smoking after OLT. Smoking behaviour was defined by using a questionnaire about smoking habits at 4 time points before and after OLT. Data on the disease course after OLT were collected from the medical charts.

Finally the results of the studies as described in this thesis are summarized and future perspectives are given in chapter 8.

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Chapter 2

Active and passive smoking behaviour and cessation plans of patients with Crohn's disease and ulcerative colitis

F. van der Heide, A. Dijkstra,
F.A. Albersnagel, J.H. Kleibeuker, G. Dijkstra

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Abstract

Introduction:
Smoking is a remarkable risk factor in inflammatory bowel disease (IBD), with negative effects on Crohn's disease (CD) and positive effects on ulcerative colitis (UC). This makes different changes in smoking behaviour after diagnosis between CD and UC likely. Changes in active smoking, cessation plans and passive smoking were studied in IBD patients.

Methods:
820 IBD patients were sent a questionnaire on active and passive smoking, and cessation plans. A total of 675 (82%) patients (380 CD and 295 UC) responded.

Results:
More ever smoking UC patients stopped smoking before diagnosis than CD patients (63% vs 22%; $p<0.001$), resulting in 30% former smokers at diagnosis in UC and 13% in CD ($p<0.001$). The smoking cessation rates at and after diagnosis are equal between CD and UC. Half of the CD patients stopped smoking after diagnosis leading to less present smokers in CD than in a control population (26% (95% confidence interval: 21.1%-29.9%) vs 33%). For both CD (22% vs 35%; $p=0.044$) and UC (24% vs 53%; $p=0.024$) continuing smokers after diagnosis were less often higher educated than quitters. Cessation plans (89%), passive smoking in childhood and present passive smoking were not different between CD and UC patients.

Conclusion:
There are no differences in changes in smoking behaviour at and after diagnosis between CD and UC patients, suggesting a lack of knowledge in these patients about the link between their disease and smoking behaviour. However, CD patients seem less refractory to smoking cessation than the general population. Therefore it is worthwhile putting energy in helping CD patients stop smoking.

Introduction

The chronic inflammatory bowel diseases (IBD) Crohn's disease (CD) and ulcerative colitis (UC) are characterized by relapsing inflammation of the gastrointestinal tract. They share many clinical features, but there are also important differences, like the disease location and histological features. Another remarkable difference is the inverse effect of smoking on CD and UC. CD patients are more likely to be smokers than their matched controls, suggesting that smoking is a risk factor for development of CD.[1-3] In contrast, UC patients are less likely to be smokers than controls, so smoking seems to protect against development of UC.[2-4]

In addition to the effect of smoking on the development of CD and UC, smoking also plays an important role on the disease course of both diseases. In CD, smokers more often experience flare-up episodes, have an increased need for steroids and immunosuppressants, and earlier recurrence after surgery.[5-11] In UC, smokers experience less flare-up episodes, and need less often hospitalizations, steroids and surgery.[11-15] The unfavourable effects of smoking on the disease course of CD patients make it very desirable for this category to stop smoking. Consequently, reviews have focussed on smoking cessation in CD,[16,17] but to the best of our knowledge there are no studies about long term changes in smoking behaviour in CD patients after diagnosis. Furthermore, since smoking has beneficial effects in UC, smoking cessation in UC patients could lead to an increase in bowel complaints resulting in smoking relapse. Summarizing, smoking cessation is beneficial for CD patients, but could lead to unfavourable effects in UC patients. If CD and UC patients are aware of this link between their disease and smoking behaviour, different changes in smoking behaviour after diagnosis between CD and UC patients are likely.

As for active smoking, there could also be a role for passive smoking in IBD. Passive smoking in childhood could be a risk factor for the development of IBD, but literature about this is scarce and inconclusive. Some studies showed that passive smoking in childhood was a risk factor for developing CD.[18,19] A recent meta-analysis showed no effect of passive smoking in childhood on CD, although a subanalysis only based on effects of maternal smoking showed a positive association with CD (odds ratio 1.3 (95% confidence interval (CI): 1.1-1.6)).[20] In UC, a protective effect of passive smoking in childhood was suggested,[21] but this could not be confirmed in a meta-analysis.[20] Studies about present passive smoking in adult IBD patients are even scarcer, with one study that showed no difference in passive smoking habits between CD and UC patients, and between these patients and controls.[22]

The aim of this study was to extensively explore active smoking behaviour and passive smoking in CD and UC patients in a Dutch university hospital population. For active smoking behaviour we focussed on changes after diagnosis of IBD and cessation plans. For passive smoking we studied differences in passive smoking in childhood and present passive smoking between CD and UC. We hypothesized that CD patients more often stop smoking and avoid passive smoking after diagnosis than UC patients, because CD patients become aware of the risks of smoking for their disease and/or UC patients experience more complaints after smoking cessation. Secondly, we hypothesize that CD patients are more often exposed to passive smoking in childhood than UC patients.

Methods

Patient Population

All IBD patients who visited the outpatient department of our hospital between January 1995 and October 2005, and had a known or incident diagnosis of CD or UC by defined criteria were included.[23] A total of 820 alive IBD patients were identified and received a detailed questionnaire about smoking behaviour. A total of 675 patients responded to the questionnaire (82%) and were included in this study. Of these 675 patients we extensively described disease characteristics and behaviour before.[15] Clinical characteristics including information about active smoking were obtained through analysis of the medical charts. Detailed information about active and passive smoking behaviour was obtained through a written questionnaire. We compared the present smoking behaviour of CD and UC patients with a general, age adjusted Dutch population from the StatLine databank of the Dutch central agency for statistics (Statistics Netherlands;http://statline.cbs.nl). The methods used were discussed with the medical ethics committee and according to Dutch legislation there were no objections against it. A returned and refilled questionnaire was considered as an informed consent.

Characteristics and outcome variables

Patient characteristics recorded were gender, age at diagnosis (divided according to the Montreal-classification in below 16 years, between 17 and 40 years, and above 40 years),[24] and time of diagnosis. Time of diagnosis was the date of the first detection of inflammatory abnormalities by radiological, endoscopic or peroperative examinations.[23] The end of follow-up was October 2005, or the last date of clinical or outpatient visit for patients who were discharged or had withdrawn from outpatient control.

Questionnaire for smoking behaviour

The written and detailed questionnaire about smoking behaviour included questions about 1) whether the patient had ever been smoking and if so, number of years smoked and, if a former smoker, months after cessation; 2) product smoked and average amount smoked per day; 3) the influence of the diagnosis of IBD on smoking behaviour; 4) plans for smoking cessation;[25] 5) willingness to participate in a free smoking cessation program; 6) passive smoking in childhood; 7) present passive smoking and 8) educational level. For studying passive smoking in childhood patients were asked whether he or she inhaled smoke from cigarette smokers at home being a child. For present passive smoking was asked how many times the patient was more than one hour in a room with smoking persons (every day, a few times a week, once a week or never).

Definitions of active smoking behaviour

Smoking behaviour was determined at the time of diagnosis and at the time of completing the questionnaire (present smoking). At both time points patients were divided in smokers, former smokers and never smokers. For defining smoking behaviour at the time of diagnosis, we used the information about smoking behaviour and number of years smoked or number of months after smoking cessation, and the time of diagnosis from the medical chart. A *smoker at diagnosis* started smoking seven or more cigarettes per week at least six months before diagnosis, a *former smoker* quitted smoking at least six months before diagnosis and

a *never smoker* had never smoked until six months before diagnosis. The average amount smoked per day was adjusted by product smoked. Smoking 1 cigar was considered equal to 4 cigarettes, as the average cigar contains 4 g of tobacco and a cigarette 1 g.[26]

Statistical analysis

Descriptive variables are presented as medians (interquartile range (IQR)) and categorical variables as frequencies with percentages. The data were analyzed using the Statistical Package for the Social Sciences program version 12.0 (SPSS Inc., Chicago, Illinois, USA). Frequencies were compared with the chi-square test en medians with the Mann-Whitney test. Differences are considered significant when $p<0.05$. The 95% CI was calculated if appropriate.

Results

Patient characteristics and smoking behaviour at diagnosis

Of the 675 patients that responded to the questionnaire (82%), 380 (56%) were known with CD and 295 (44%) with UC (table 1). CD patients were younger at diagnosis, more often female and had a longer follow-up than UC patients. CD patients were more often smokers and UC patients were more often both former and never smokers at diagnosis. In a previous study we already confirmed the difference in active smoking behaviour at diagnosis between CD and UC patients, and between these patients and the general population, with 52% (95%CI: 47.1%-57.2%) smokers in CD, 41% smokers in the general population and 28% (95%CI: 22.7%-32.9%) smokers in UC.[15]

Active smoking after diagnosis and influence of the diagnosis on smoking behaviour

To study changes in active smoking behaviour after diagnosis in CD and UC, we explored the influence of the diagnosis on the smoking behaviour and the present smoking behaviour (table 2). We hypothesized that when CD patients are aware of the risks of smoking for their disease and/or UC patients experience more complaints during smoking cessation, than after diagnosis CD patients more often have stopped smoking than UC patients.

First, ever smokers (smokers and former smokers) at diagnosis were asked about the influence of the diagnosis of IBD on their smoking behaviour. UC patients more often had already stopped smoking before the diagnosis than CD patient. Smoking cessation rate at diagnosis was not different between CD and UC patients, although CD patients more often reduced smoking after being diagnosed with the disease. Relatively more CD patients did not change their smoke behaviour at diagnosis than UC patients.

Next, present active smoking was studied. Presently CD patients are still more often smokers and UC patients are more often never smokers. Furthermore, the number of cigarettes smoked per day is higher in present CD smokers than UC smokers. We compared the number of smokers with an age adjusted control population, in which 35% were smokers in 2006. In both CD (26% (95%CI: 21.1%-29.9%)) and UC (10% (95%CI: 6.4%-13.3%)) the number of present smokers is lower. Considering the higher number of females in CD, we also adjusted the smoking behaviour of the controls to gender. This leads to 33% smokers, which is still higher than in CD patients.

Table 1 Characteristics of the respondents.

Characteristic: n (%)	CD (n=380)	UC (n=295)	p-value
Gender: female	241 (63.4)	139 (47.1)	<0.001
Age at diagnosis:			
median (yrs) (IQR)	26.7 (20.6-39.7)	32.4 (23.9-41.7)	<0.001
below 16 yrs: n (%)	32 (8.4)	17 (5.8)	ns
between 17 and 40 yrs: n (%)	254 (66.8)	193 (65.4)	ns
above 40 yrs: n (%)	94 (24.7)	85 (28.8)	ns
Period of diagnosis:			
1950-1959	3 (7.9)	3 (1.0)	
1960-1969	16 (4.2)	3 (1.0)	
1970-1979	36 (9.5)	19 (6.4)	
1980-1989	65 (17.1)	48 (16.3)	
1990-1999	150 (39.5)	152 (51.5)	
2000-2005	110 (28.9)	70 (23.7)	
Follow-up: median(yrs) (IQR)	9.4 (4.9-19.0)	8.5 (4.1-14.6)	0.008
Education:			
lower	80 (21.1)	59 (20.0)	ns
medium	150 (39.5)	121 (41.0)	ns
higher	150 (39.5)	115 (39.0)	ns
Smoking behaviour at diagnosis:			
smoker	198 (52.1)	82 (27.8)	<0.001
former smoker	48 (12.6)	87 (29.5)	<0.001
never smoker	134 (35.3)	126 (42.7)	0.049

CD: Crohn's disease; IQR: interquartile range; UC: ulcerative colitis.

Table 2 Influence of diagnosis on smoking behaviour in ever smoking CD and UC patients, and present smoking behaviour in all patients.

Characteristic: n (%)	CD (n=380)	UC (n=295)	p-value
Influence of diagnosis on smoking:			
already stopped	53/240 (22.1)	104/166 (62.7)	<0.001
stopped then	33 (13.8)	15 (9.0)	ns
started again	2 (0.8)	6 (3.6)	0.047
started smoking less	43 (17.9)	5 (3.0)	<0.001
started smoking more	6 (2.5)	0	0.040
no influence	103 (42.9)	36 (21.7)	<0.001
Present smoking behaviour:			
smoker	97 (25.5)	29 (9.8)	<0.001
former smoker	156 (41.1)	141(47.8)	ns
never smoker	127 (33.4)	125 (42.4)	0.017
Cigarettes per day: median (IQR)			
present active smokers	12.0 (7.3-20.0)	10.0 (4.3-12.4)	0.007
present former smokers	12.3 (6.0-20.0)	12.5 (8.0-20.0)	ns

CD: Crohn's disease; IQR: interquartile range; UC: ulcerative colitis.

Finally, to study differences in smoking cessation rates after diagnosis between CD and UC patients, we analyzed whether smokers at diagnosis were presently still smoking. Here to we excluded the smokers at diagnosis who stopped smoking within one year after diagnosis, because of our relatively rough measures of smoking behaviour at diagnosis. After this exclusion, 91 of the 179 (51%) CD patients and 38 of the 63 (60%) UC patients had stopped smoking after diagnosis. This difference is not significant (p=0.195). In addition, differences between the continuing smokers and the smokers that stopped smoking after diagnosis were studied. For CD, the continuing smokers were more often lower (34% vs 21%; p=0.047) and less often higher (22% vs 35%; p=0.044) educated than quitters, and smoked less often cigarettes (53% vs 78%; p=0.001) and cigars (1% vs 8%; p=0.034) than quitters. For UC, the continuing smokers were less often higher educated (24% vs 53%; p=0.024) than quitters and smoked fewer cigarettes per day (median (IQR): 10.0 (5.0-12.5) vs 12.5 (9.3-20.0); p=0.005) than quitters. For both diseases no differences were found for gender, age at diagnosis, onset of disease and passive smoking in childhood.

Summarizing, UC patients more often stopped smoking before diagnosis and the cessation rate at diagnosis is similar. In contrast to our hypothesis, CD patients did not stop smoking more often after diagnosis than UC patients. However, still half of the CD patients have managed smoking cessation after diagnosis leading to a lower number of present smokers in CD than in a control population. In both CD and UC, a higher education is associated with smoking cessation after diagnosis.

Smoking cessation plans

To explore our hypothesis that CD patients are more willing to quit smoking compared to UC patients, we studied the smoking cessation plans and willingness to participate in a smoking cessation program for present smoking CD and UC patients (table 3). We could not confirm our hypothesis that there are differences between CD and UC patients in smoking cessation plans and willingness to join a group course for smoke cessation. However, for both diseases about 90% had the intention to stop smoking, although less than 30% wanted to join a free course for smoking cessation.

Table 3. Smoking cessation plans and intention to join a group course for cessation in smoking CD and UC patients.

Characteristic: n (%)	CD (n=97)	UC (n=29)	p-value
Cessation plan:			
within 6 months	32/94 (34.0)	9/27 (33.3)	ns
within 5 years	30 (31.9)	10 (37.0)	ns
once	22 (23.4)	5 (18.5)	ns
never	10 (10.6)	3 (11.1)	ns
Group course for cessation:			
yes	24/94 (25.5)	4 (13.8)	ns
maybe	40 (42.6)	12 (41.4)	ns
no	30 (31.9)	13 (44.8)	ns

CD: Crohn's disease; UC: ulcerative colitis.

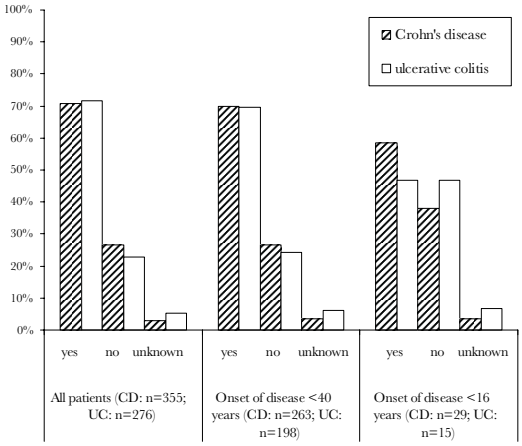

Figure 1. Passive smoking in childhood in Crohn's disease (CD) and ulcerative colitis (UC) patients, with no significant differences between CD and UC. Numbers are shown for all patients, for patients with an onset of disease <40 years, and for patients with an onset of disease <16 years.

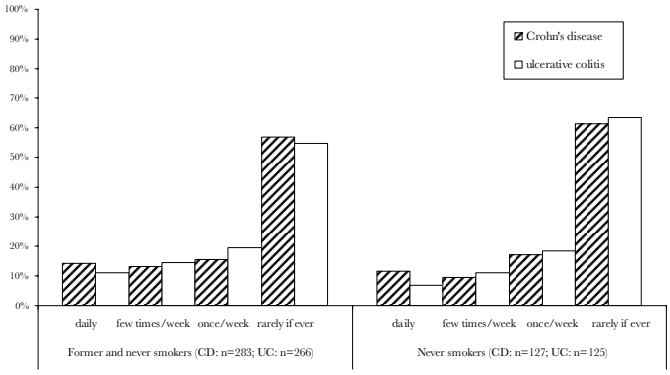

Figure 2. Present passive smoking in Crohn's disease (CD) and ulcerative colitis (UC), with no significant differences between CD and UC. Numbers are shown for present non-smokers (former and never smokers) and for present never smokers.

Passive smoking in childhood

To study the relation between passive smoking and the development of IBD, we explored differences in passive smoking in childhood between CD and UC. We studied this in all patients, in patients with onset of disease before 40 years and with onset of disease before 16 years (Fig. 1). In all these groups passive smoking in childhood between CD and UC patients did not differ significantly.

Present passive smoking behaviour in non-smokers

Finally, we studied differences in present passive smoking between CD and UC. We studied this in present non-smokers (former and never smokers) to exclude a confounding effect of present active smoking. The present passive smoking in non-smoking CD and UC patients is shown in figure 2, for both former and never smokers combined (non-smokers), and never smokers

alone. In disagreement with our hypothesis that CD patients are aware of the risks of smoking for their disease and therefore more often avoid passive exposure to smoke than UC patients, we could not show significant differences in present passive smoking between CD and UC.

Discussion

Smoking is a remarkable and well established risk factor in IBD, with negative effects on CD and positive effects on UC. The inverse effects of smoking on CD and UC makes differences in changes in smoking behaviour after diagnosis between CD and UC likely, but hitherto no such information was available. Therefore, we extensively studied changes in active smoking, cessation plans and passive smoking in CD and UC patients in a large, Dutch university hospital population. The smoking cessation rate before diagnosis is higher in UC, but cessation rates at diagnosis and after diagnosis are equal between CD and UC. Half of the CD patients have managed smoking cessation after diagnosis leading to a lower number of present smokers in CD than in a control population. Cessation plans and willingness to join a cessation course are not different between CD and UC patients. Passive smoking in childhood and present passive smoking were studied, and both were not different between CD and UC.

In a previous analysis of this IBD population we already confirmed the difference in active smoking behaviour at diagnosis between CD and UC patients, and between these patients and a control population, with 52% (95%CI: 47%-57%) smokers in CD, 41% smokers in the controls and 28% (95%CI: 23%-33%) smokers in UC.[15] In the present analysis, it was shown that in ever smoking IBD patients, 63% of ever smoking UC patients stopped smoking before diagnosis compared to only 22% of CD patients, resulting in 30% former smoking patients in UC and 13% in CD at diagnosis. These numbers of former smokers are comparable with the numbers Aldhouse et al. reported, although they defined former smokers as those who had stopped smoking one year or more before diagnosis instead of the six months in our study.[27,28] This striking difference in former smokers between CD and UC is supportive for a causal relationship between smoking cessation and developing UC.

We hypothesized that CD patients more often stop smoking after diagnosis and avoid passive smoking after diagnosis than UC patients, because CD patients become aware of the risks of smoking for their disease and/or UC patients experience more complaints after smoking cessation. However, our results are in contradiction with this. First, more CD patients reported no influence of the diagnosis on smoking behaviour than UC patients (43% vs 22%) and smoking cessation at diagnosis was not different between CD and UC patients (14% vs 9%). Next, CD patients did not more often stop their smoking after diagnosis than UC patients (51% vs 60%) and CD patients did not more often avoid passive exposure to cigarette smoke. Finally, the present cessation plans are also not different between CD and UC patients; in both diseases about 35% have the intention to stop within six months. Not only are there no clear differences in changes in smoking behaviour between CD and UC patients, presently CD patients smoke more cigarettes per day than UC patients (12 vs 10).

Fortunately, there are also some positive findings in CD patients. First, CD patients more often reduced smoking at diagnosis than UC patients (18% vs 3%). Next, half of the CD patients have managed smoking cessation after diagnosis leading to a lower number of present

smokers in CD than in the controls. Finally, in both diseases only 11% of the patients have no intention to stop smoking. This number is favourable compared to that in a group of 6260 healthy smokers from Australia, Canada, USA and UK in which 26% had no intention to stop smoking.[29] Therefore, both the intention to stop and the cessation rate in CD patients is better than in the general population. Thus, CD patients are less refractory to smoking cessation than the general population and therefore it is absolutely worthwhile to put energy in helping CD patients stop smoking. This is more or less in line with a previous study, that did not find evidence that smoking CD patients were overly refractory to smoking cessation.[30] Reviews that could be helpful in assisting CD patients with smoking cessation are available.[16,17] Furthermore, it is important to keep in mind that CD patients could be less aware of the risks of smoking on their disease than we think, since studies showed an unawareness in CD patients of the association between smoking and their disease.[31,32] Another issue to keep in mind is that level of education plays an important role in smoking cessation in the general population.[33,34] We confirmed this in both CD and UC patients, since patients who succeeded in smoking cessation after diagnosis more often had completed higher education. Cosnes et al. showed that high socioeconomic status was a predictor of smoking cessation for more than one year in their intervention study.[35]

In addition to changes in active smoking behaviour and present passive smoking, we also studied differences in passive smoking in childhood between CD and UC patients. Some studies have suggested that passive smoking in childhood could also have effects on the development of CD and UC,[18,19,21] but a recent meta-analysis showed no differences in passive smoking in childhood between both CD and UC, and controls.[20] Our findings of an equal rate in passive smoking in childhood between CD and UC patients are in line with this, although we did not include a control population. However, the effects of passive smoking in childhood could be dose-dependent [20,36,37] and therefore more studies are needed that analyze the effects of different levels of passive smoking in childhood on IBD.

It is important to note limitations of our study. Using a questionnaire may lead to selection bias, because it takes more time for smokers and/or former smokers to fill in the survey and they could be less willing to respond. However, the high response-rate to our questionnaire (82%) makes this unlikely. Asking about smoking behaviour in the past and the influence of the diagnosis of the disease on smoking behaviour could introduce a recall bias. The data on passive smoking in childhood was not confirmed by asking the parents of the patients. However, using a written questionnaire gives patients time to ask their parents about it.

In conclusion, we extensively studied changes in active smoking, cessation plans and passive smoking in CD and UC patients. The smoking cessation rate before diagnosis is higher in UC, but cessation rates at and after diagnosis are equal among CD and UC patients. In addition, there are no indications that CD patients avoid passive exposure to smoke more often. These findings suggest a lack of knowledge in CD and UC patients about the link between their disease and smoking behaviour, although our questionnaire did not address this knowledge in particular. However, half of the CD patients have managed smoking cessation after diagnosis leading to a lower number of present smokers in CD than in a control population, and many CD patients have cessation plans. So, CD patients are less refractory to smoking cessation than the general population and therefore it is worthwhile to put energy in helping CD patients stop smoking. In both CD and UC, a higher education is associated with smoking cessation after diagnosis. Finally, passive smoking in childhood was not different between CD and UC, but further research on this matter is needed.

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Chapter 3

Effects of active and passive smoking on disease course of Crohn's disease and ulcerative colitis

F. van der Heide, A. Dijkstra, R.K. Weersma, F.A. Albersnagel, E.M.J. van der Logt, K.N. Faber, W.J. Sluiter, J.H. Kleibeuker, G. Dijkstra

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Abstract

Introduction:
Smoking is a remarkable risk factor for inflammatory bowel disease (IBD), aggravating Crohn's disease (CD) while having beneficial effects on ulcerative colitis (UC). We studied the effects of active and passive smoking in Dutch IBD patients.

Methods:
A questionnaire focussing on cigarette smoke exposure was sent to 820 IBD patients. Returned questionnaires were incorporated into a retrospective chart review, containing details about disease behaviour and received therapy.

Results:
675 IBD patients (380 (56%) CD and 295 (44%) UC) responded. At diagnosis there were 52% smokers in CD, 41% in the general population and 28% in UC. The number of present smokers in CD is lower than in the general population (26% vs. 35%). No detrimental effects of active smoking on CD were observed, but passive smokers needed more frequently immunosuppressants and infliximab than non-passive smokers. Active smoking had beneficial effects on UC, indicated by reduced rates of colectomy, primary sclerosing cholangitis and backwash-ileitis in active smokers compared to never smokers, and higher daily cigarette-dose correlated with less extensive colitis and lower need for therapy. Furthermore, smoking cessation after diagnosis was detrimental for UC patients, indicated by increased needs for steroids and hospitalizations for patients that stopped smoking after compared to before the diagnosis.

Conclusion:
Active smoking is a risk factor for CD, but does not affect the outcome; passive smoking is detrimental for the outcome of CD patients. In UC, active smoking shows dose-dependent beneficial effects. Our data suggest that passive smoking is a novel risk factor for CD.

Introduction

Chronic inflammatory bowel diseases (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), are characterized by chronic relapsing inflammation of the gastrointestinal tract. They share many clinical features, but there are also important differences, like the disease location and histological features. Another remarkable difference is the effect of smoking on CD and UC. Several studies have shown that patients with UC are less likely to be smokers than controls or patients with CD.[1] In contrast, CD patients are more likely to be smokers than their matched controls.[2-5] So smoking seems to be a contributing factor for the development of CD, while it protects against the development of UC.

Besides the association between smoking and the prevalence of IBD, several studies showed an unfavourable effect of smoking on the disease course of CD and a beneficial effect on that of UC. Smoking in CD was associated with more flare-up episodes,[6] more complications,[7] increased need for steroids[6] and immunosuppressants[6,8,9] and earlier recurrence after surgery.[10-12] However, other studies did not confirm these findings.[13-20] In contrast to the unfavourable effect of smoking in CD, in UC smoking was associated with less flare-up episodes,[21] lower number of hospitalizations,[14-16,18,21] and decreased need for oral steroids[22] and surgery.[18,23] However, the positive effect of smoking on colectomy rate was not shown in other cohorts.[14,21,24,25] So studies about the role of smoking on the disease course of CD as well as UC give conflicting results, and effects of smoking seem to depend on gender,[8,26] disease location and severity.[27] Furthermore, like the effects of active smoking on IBD, passive smoking may also affect the course of IBD. However, very little is known on the effect of passive smoking on the disease course of IBD.

The aim of this study was to analyze the relationship between smoking (active and passive smoking and smoking cessation) and the course and behaviour of CD and UC over a mean time frame of 13 years in a Dutch IBD cohort at a university hospital. We hypothesized that 1) both active and passive smoking are detrimental for the disease course of CD and 2) both active and passive smoking are beneficial for the disease course of UC.

Methods

Patients

IBD patients who visited the outpatient department of our hospital between January 1995 and October 2005 and had a confirmed diagnosis of CD or UC by endoscopic, radiologic or pathologic examinations[28] were included. Patients are seen at a regular basis and more often if needed at our outpatient department. Patients with indeterminate colitis or a concomitant liver transplantation were excluded. A total of 820 IBD patients alive were identified. Detailed information about smoking behaviour was obtained through a written questionnaire. Smoking behaviour at diagnosis (median year of diagnosis was 1995) of CD and UC patients were compared with 1995-records of a general, age adjusted Dutch population of the StatLine databank of the Dutch central agency for statistics (Statistics Netherlands;http://statline.cbs.nl). Clinical characteristics and the outcome variables were obtained through retrospective analysis of the medical charts. The methods used were discussed with the medical ethics committee. According to Dutch legislation there were no objections against the methods used. A returned questionnaire was considered as an informed consent.

Questionnaire for smoking behaviour

All patients received a detailed questionnaire about smoking behaviour. This included questions about 1) whether the patient had ever been smoking and if so, number of years smoked and, if a former smoker, months after cessation; 2) product smoked and average amount smoked per day; 3) the relation between diagnosis and smoking behaviour; 4) passive smoking at present (every day, a few times a week, once a week or never/seldom more than one hour a day in the same room with smoking persons); 5) educational level and 6) family history of IBD.

Definitions of smoking behaviour

Smoking behaviour was determined at the time of diagnosis and at the time of completing the questionnaire (present smoking), and patients were divided in smokers, former smokers and never smokers. A *smoker at diagnosis* started smoking seven or more cigarettes per week at least six months before diagnosis, a *former smoker* quitted smoking at least six months before diagnosis and a *never smoker* had never smoked until six months before diagnosis.

For studying the relation between smoking and disease course, patients were divided in: 1) *smokers after diagnosis* (smoker at diagnosis who did not stop smoking within one year after diagnosis, or former smoker at diagnosis who restarted smoking within one year after diagnosis, or never smoker at diagnosis who smoked within one year after diagnosis), 2) *quitters after diagnosis* (smoker at diagnosis who stopped smoking within one year after diagnosis), 3) *quitters before diagnosis* (former smoker at diagnosis who did not restart smoking within one year) and 4) *never smokers after diagnosis* (never smoked until one year after diagnosis).

The level of smoke exposure was analyzed in smokers after diagnosis by dividing the patients in three groups according to the number of cigarettes smoked per day (<10, 10-15, >15 per day). Smoking of one cigar was considered equal to four cigarettes, as the average cigar contains four grams of tobacco and a cigarette one gram.[29] For studying the relation between passive smoking and disease course, never smokers were divided in two groups: 1) passive smokers (daily, few times a week or once a week exposed to a smoking environment) and 2) non-passive smokers (rarely or never exposed).

Clinical characteristics and outcome variables

Patient characteristics recorded were gender, age at diagnosis (divided according to the Vienna-classification in early age onset (< 40 years) and late onset disease (> 40 years)),[30] time of diagnosis and type of disease (CD or UC). The time of diagnosis was defined as the date of the first detection of inflammatory abnormalities by radiological, endoscopic or peroperative examinations. For CD, disease behaviour and location were determined according to the Vienna-classification.[30] Behaviour was determined at diagnosis and during follow-up. For UC, location was defined as proctitis, left-sided colitis (not extending beyond the splenic flexure) and pancolitis with or without backwash-ileitis, defined as mucosal inflammation of the terminal ileum as a continuation of colonic involvement, without histological features of CD. We determined the location at diagnosis and the maximal extend during follow-up. Extraintestinal manifestations (EIM), including primary sclerosing cholangitis (PSC), were noted.

We recorded the use of 5-aminosalicylates (5-ASA) (oral and topical), steroids (oral, topical and iv), azathioprine or 6-mercaptopurine, methotrexate, cyclosporine and infliximab at any time during follow-up. For infliximab we recorded the indication (luminal or fistulizing disease) and response to induction or maintenance therapy according to a previously

reported three point scale.[31] Surgery for CD was defined as any intra-abdominal surgical procedure for treatment of CD or its complications, incision and drainage of a perianal abscess, and surgical treatment of perianal fistula. For UC surgery was defined as a (sub) total colectomy. Furthermore, construction of a pouch and development of pouchitis were recorded. Hospitalizations were defined as any admission for a flare-up of the disease, for a surgical procedure as outlined above or for non-surgical treatment of a complication due to stricturing or penetrating disease behaviour. Hospitalizations for scheduled infliximab infusions were excluded.

All events were recorded till the end of follow-up, October 2005. The last date of clinical or outpatient control visit was considered the end of follow-up for patients who were discharged or had withdrawn from outpatient control. For UC patients after a colectomy and without development of pouchitis, the date of colectomy was the end of follow-up.

Statistical analysis

Descriptive variables are presented as medians (interquartile range (IQR)) and categorical variables as frequencies with percentages. The data were analyzed using the Statistical Package for the Social Sciences program version 12.0 (SPSS Inc., Chicago, Illinois, USA). Frequencies were compared with the chi-square test and means between two groups with the Mann-Whitney test and between three or more groups with the Kruskal-Wallis test. Differences are considered significant when $p \leq 0.05$. All patient characteristics that showed a p-value ≤ 0.10 in univariate analysis were included in the Cox-regression analysis for multivariate analysis with follow-up as time variable. The odds ratios (OR) and the 95% confidence intervals (95% CI) were calculated if appropriate.

Results

Patients characteristics and smoking behaviour

From the 820 patients, 675 patients responded to the questionnaire (82%), with 380 (56%) CD and 295 (44%) UC patients. Of the responders, demographic characteristics, smoking behaviour (at diagnosis and present), frequency of EIM, Vienna-classification for CD, and location at diagnosis and maximal location for UC are shown in Table 1. CD patients were more often female and younger at diagnosis, and had a longer follow-up than UC patients. At diagnosis CD patients were more often smokers and UC patients were more often former smoker as well as never smokers. At the median time point of diagnosis (1995) 41% were smokers in the control population. For CD this gives an OR of 1.27 (95%CI: 1.15-1.40) and for UC 0.68 (95%CI: 0.55-0.80) for being a smoker at diagnosis. At present, CD patients are still more often smokers than UC patients and UC patients are more often never smokers. However, the number of present active smokers in CD (26% (95%CI: 21.1%-29.9%)) was lower compared to the control population, in which 35% were smokers in 2006. This gives for CD an OR of 0.74 (95%CI: 0.60-0.85) and for UC 0.29 (95%CI: 0.18-0.38) for being a present smoker.

Table 1 Characteristics of the respondents with CD and UC

Characteristic: n (%)	CD (n=380)	UC (n=295)	p-value
Gender: female	241 (63.4)	139 (47.1)	<0.001
Age at diagnosis:			
median (yrs) (IQR)	26.7 (20.6-39.7)	32.4 (23.9-41.7)	<0.001
early onset: n (%)	286 (75.3)	210 (71.2)	ns
late onset: n (%)	94 (24.7)	85 (28.8)	ns
Follow-up: median (yrs) (IQR)	9.4 (4.9-19.0)	8.5 (4.1-14.6)	0.008
Lost to follow-up	32 (8.4)	44 (14.9)	
Education:			
lower	80 (21.1)	59 (20.0)	ns
medium	150 (39.5)	121 (41.0)	ns
higher	150 (39.5)	115 (39.0)	ns
Family history of IBD:			
yes	111 (29.2)	74 (25.1)	ns
no	262 (68.9)	211 (71.5)	ns
unknown	7 (1.8)	10 (3.4)	ns
Smoking behaviour at diagnosis:			
smoker	198 (52.1)	82 (27.8)	<0.001
former smoker	48 (12.6)	87 (29.5)	<0.001
never smoker	134 (35.3)	126 (42.7)	0.049
Present smoking behaviour:			
smoker	97 (25.5)	29 (9.8)	<0.001
former smoker	156 (41.1)	141(47.8)	ns
never smoker	127 (33.4)	125 (42.4)	0.017
EIM	62 (16.3)	39/292 (13.4)	ns
PSC	5 (1.3)	21 (7.1)	<0.001
Location CD:			
terminal ileum	123 (32.4)		
colon	98 (25.8)		
ileocolon	135 (35.5)		
upper GI-tract	24 (6.3)		
Behaviour at diagnosis CD*:			
B1	260 (70.3)		
B2	48 (13.0)		
B3	62 (16.8)		
Behaviour during follow-up CD*:			
B1	128 (33.7)		
B2	103 (27.1)		
B3	149 (39.2)		
Location at diagnosis UC:			
proctitis		114/288 (39.6)	
left-sided		104 (36.1)	
pancolitis		65 (22.6)	
backwash-ileitis		5 (1.7)	
Maximal location UC:			
proctitis		34/293 (11.6)	
left-sided		110 (37.5)	
pancolitis		136 (46.4)	
backwash-ileitis		13 (4.4)	

*: B1= non-stricturing, non-penetrating; B2= stricturing; B3= penetrating.
CD: Crohn's disease; EIM: extraintestinal manifestations; IQR: interquartile range; PSC: primary sclerosing cholangitis; UC: ulcerative colitis.

Patient characteristics according to smoking behaviour at diagnosis

In CD, never smokers were the youngest at diagnosis, followed by smokers, whereas former smokers were the oldest at diagnosis (22.4 (IQR 16.7-30.9) vs. 28.3 (IQR 22.0-37.9) vs. 43.4 (IQR 31.4-54.3) years; $p<0.001$). Disease behaviour at diagnosis was similar for smokers, former smokers and never smokers. In UC, never smokers were the youngest at diagnosis, followed by smokers, whereas former smokers were the oldest at diagnosis (24.9 (IQR 20.3-32.8) vs. 32.1 (IQR 25.4-38.4) vs. 42.4 (IQR 34.6-52.9) years; $p<0.001$). Smokers had a similar disease localisation at diagnosis as former and never smokers, but never smokers presented more often with a pancolitis (27% vs. 15%; $p=0.048$) and less often with a left-sided colitis at diagnosis (29% vs. 48%; $p=0.006$) than former smokers.

Disease outcome in Crohn's disease

Active smoking

The relation between smoking behaviour and outcome variables in CD is depicted in Table 2. Smoking CD patients used more often oral 5-ASA, but despite the hypothesized negative effect of smoking, there were no differences between smokers and never smokers for the other outcome variables. We observed no differences in outcome between quitters after and before diagnosis, except that ileocolonic disease was more frequent in quitters after diagnosis. Quitters after diagnosis less often had a change in disease behaviour than smokers. Quitters before diagnosis had less hospitalizations, surgery and ileocolonic disease, and a more beneficial disease behaviour than smokers. However, the follow-up of both quitters after and before diagnosis is shorter than for smokers. Less EIM were experienced by smokers than never smokers and by quitters before diagnosis than quitters after diagnosis.

So no main differences between the outcome of smokers and never smokers were shown. It is possible that these findings are biased by our definition of smokers after diagnosis (no smoking cessation within one year after diagnosis), because a patient that stopped smoking two years after diagnosis is nevertheless called a smoker after diagnosis. Some of our so-called smokers after diagnosis probably have stopped smoking more than a year after diagnosis, since our data show a drastic decline in active smokers between time of diagnosis and end of follow-up (from 52% to 26%). Therefore, we composed a subgroup of patients that smoked during the complete follow-up and compared them with the never smokers. This subgroup of smokers received more often iv steroids than never smokers (16% vs. 6%; $p=0.010$), but there were no differences for the other outcome variables. In addition, no differences in outcome between smokers and never smokers were found in female CD patients, including female CD patients with ileal disease. Furthermore, the outcome of treatment of luminal or fistulizing disease with infliximab did not differ between smokers, quitters and never smokers (data not shown).

In CD no dose-effect of smoking in smokers after diagnosis could be established on one of the outcome variables (data not shown).

Passive smoking

Since several studies have shown an unfavourable effect of active smoking on the course of CD, we also studied the effects of passive smoking. We divided never smoking CD patients in passive and non-passive smokers. Even with a shorter follow-up (median 7 years vs. 13 years; $p=0.002$), passive smokers ($n=56$) needed more immunosuppressants (68% vs. 49%; $p=0.039$) and infliximab (29% vs. 11%; $p=0.013$) compared to non-passive smokers ($n=65$). No effect of passive smoking was observed for the other outcome variables (data not shown).

Table 2. Outcome variables for Crohn's disease according to smoking behaviour after diagnosis

Characteristic (%)	Smokers (n=166)	Quitters after diagnosis (n=33)	Quitters before diagnosis (n=48)	Never smokers (n=131)
Follow-up: median (yrs) (IQR)	12.1 (6.8-20.5)	4.9 (2.4-16.5)*<0.001	6.0 (2.6-11.1)*<0.001	9.4 (4.9-18.8)#0.007 †0.001
Hospitalizations:				
at least one	130/163 (79.8)	22 (66.7)	28 (58.3)*0.003	99/130 (76.2)†0.020
at least two	96/163 (58.9)	17 (51.5)	16 (33.3)*0.002	73/130 (56.2)†0.007
Surgery:				
at least one	107 (64.5)	19 (57.6)	20 (41.7)*0.005	74 (56.5)
at least two	62 (37.3)	7 (21.2)	13 (27.1)	36 (27.5)
No therapy	2/165 (1.2)	1 (3.0)	1 (2.1)	5 (3.8)
5-ASA:	154/165 (93.3)	25/32 (78.1)*0.006	38/47 (80.9)*0.010	110/129 (85.3)*0.023
topical	24/162 (14.8)	4/32 (12.5)	11/47 (23.4)	16/128 (12.5) 110/129
oral	153/165 (92.7)	25/32 (78.1)*0.010	37/47 (78.7)*0.005	(85.3)*0.039
Steroids:	133/162 (82.1)	24 (72.7)	39/46 (84.8)	106/129 (82.2)
topical	38/162 (23.5)	8 (24.2)	12/46 (26.1)	22/128 (17.2)
oral	130/162 (80.2)	22 (66.7)	38/46 (82.6)	106/129 (82.2)
iv	20/162 (12.3)	2/32 (6.3)	6/46 (13.0)	8/127 (6.3)
IS¶	100 (60.2)	15 (45.5)	25 (52.1)	74 (56.5)
Infliximab	34 (20.5)	4 (12.1)	9 (18.8)	25 (19.1)
EIM	21 (12.7)	8 (24.2)	4 (8.3)# 0.048	29 (22.1)*0.030 †0.035
PSC	1 (0.6)	0	1 (2.1)	3 (2.3)
Location:				
ileum	55 (33.1)	9 (27.3)	19 (39.6)	40 (30.5)
colon	38 (22.9)	9 (27.3)	15 (31.3)	35 (26.7)
ileocolon	63 (38.0)	14 (42.4)	10 (20.8)* 0.028 # 0.037	47 (35.9)
upper GI-tract	10 (6.0)	1 (3.0)	4 (8.3)	9 (6.9)
Behaviour at diagnosis§:				
B1	113 (72.0)	21 (63.6)	34 (70.8)	90 (69.2)
B2	18 (11.5)	6 (18.2)	8 (16.7)	16 (12.3)
B3	26 (16.6)	6 (18.2)	6 (12.5)	24 (18.5)
Behaviour during follow-up§:				
B1	44 (26.5)	14 (42.4)	23 (47.9)*0.005	47 (35.9)
B2	52 (31.3)	6 (18.2)	15 (31.3)	30 (22.9)
B3	70 (42.2)	13 (39.4)	10 (20.8)*0.007	54 (41.2)†0.012
Change:				
B1 at diagnosis to B2 or B3	69/113 (61.1)	7/21 (33.3))*0.019	11/34 (32.4))*0.003	43/90 (47.8))*0.059

*: p ≤ 0.05 vs smokers; #: p ≤ 0.05 vs quitters after diagnosis; †: p ≤ 0.05 vs quitters before diagnosis.
¶: Azathioprine, 6-mercaptopurine and/or methotrexate.
§: B1= non-stricturing, non-penetrating; B2= stricturing; B3= penetrating.
5-ASA: 5-aminosalicylates; EIM: extraintestinal manifestations; IQR: interquartile range; IS: immunosuppressants; PSC: primary sclerosing cholangitis.

Disease outcome in ulcerative colitis

Active smoking

The relation between smoking behaviour and disease outcome variables in UC is depicted in Table 3. These data show a beneficial relation between active smoking and outcome in UC, except for pouchitis. Even with a longer follow-up, smoking UC patients had a lower colectomy rate, less PSC, and less backwash-ileitis than UC patients who never smoked. The development of pouchitis was not significantly different between smokers and never smokers, but patients who quitted smoking before diagnosis had less pouchitis than smokers. Quitters before diagnosis had fewer hospitalizations and needed less oral steroids than quitters after diagnosis. Quitters before diagnosis more often had left-sided colitis and less often pancolitis during follow-up than never smokers.

Cigarette-dose

A dose-dependent beneficial effect of smoking on disease outcome variables of UC was observed in smokers after diagnosis (Figure 1). Heavy smokers (>15 cigarettes/day) had less extensive disease (75% vs. 100%; p=0.023), received less oral (56% vs. 88%; p=0.032) and iv steroids (0% vs. 35%; p=0.006), used less immunosuppressants (15% vs. 47%; p=0.033), and had less hospitalizations (26% vs. 59%; p=0.048) than light smokers (<10 cigarettes/day).

Passive smoking

We divided never smoking UC patients in passive and non-passive smokers. In contrast to the beneficial effect of active smoking, passive smokers (n=43) developed more pouchitis

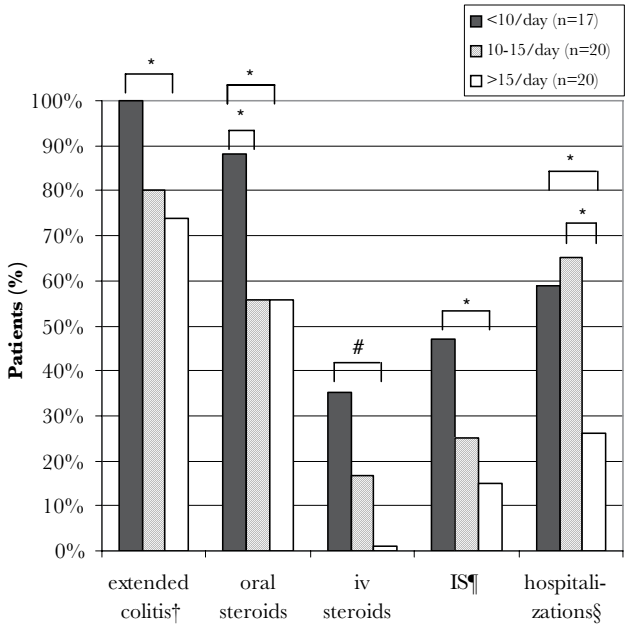

Figure 1. Daily dose-effect of active smoking in ulcerative colitis
*: p-value < 0.05; #: p-value < 0.01;
†: inflammation above proctum; ¶: Azathioprine, 6-mercaptopurine and/or methotrexate;
§: hospitalizations once or more.
IS: immunosuppressants.

Table 3 Outcome variables for ulcerative colitis according to smoking behaviour after diagnosis

Characteristic (%)	Smokers (n=58)	Quitters after diagnosis (n=24)	Quitters before diagnosis (n=86)	Never smokers (n=126)
Follow-up: median (yrs) (IQR)	11.2 (7.1-18.5)	4.9 (3.8-13.7)*0.007	6.5 (2.8-12.3)*<0.001	9.0 (4.1-15.3)*0.035 †0.037
Hospitalizations:				
at least one	29/57 (50.9)	13 (54.2)	27/85 (31.8)*0.022 #0.044	65/124 (52.4)†0.003
at least two	12/57 (21.1)	5 (20.8)	9/85 (10.6)	40/124 (32.3)†<0.001
Colectomy	7 (12.1)	5 (20.8)	13 (15.1)	35 (27.8)*0.018 †0.031
Pouch	5 (8.6)	0	5 (6.0)	15 (12.0)
Pouchitis	5/5 (100)	0	1/5 (20.0)*0.010	9/15 (60.0)
No therapy	0	0	0	0
5-ASA:	57/57 (100)	24 (100)	86 (100)	126 (100)
topical	44/54 (81.5)	19 (79.2)	71 (82.6)	102/125 (81.6)
oral	53/56 (94.6)	23 (95.8)	77 (89.5)	121 (96.0)
Steroids:	42/54 (77.8)	21 (87.5)	65/85 (76.5)	106 (84.1)
topical	33/54 (61.1)	17 (70.8)	54/85 (63.5)	92/125 (73.6)
oral	36/54 (66.7)	19 (79.2)	47/85 (55.3)#0.035	84 (66.7)
iv	9/54 (16.7)	3 (12.5)	10/85 (11.8)	19/125 (15.2)
IS¶	16 (27.6)	10 (41.7)	28 (32.6)	48 (38.1)
Cyclosporine	3 (5.2)	1 (4.2)	2 (2.3)	3 (2.4)
EIM	5/57 (8.8)	2 (8.3)	9/85 (10.6)	23/125 (18.4)
PSC	2 (3.4)	0	3 (3.5)	16 (12.7)*0.050 †0.021
Location at diagnosis:				
proctitis	24/54 (44.4)	8 (33.3)	31/85 (36.5)	51/124 (41.1)
left-sided	16 (29.6)	11 (45.8)	41 (48.2)*0.030	36 (29.0)†0.005
pancolitis	14 (25.9)	5 (20.8)	12 (14.1)	33 (26.6)†0.031
backwash-ileitis	0	0	1 (1.2)	4 (3.2)
Maximal location:				
proctitis	9/57 (15.8)	2 (8.3)	11 (12.8)	12/125 (9.6)
left-sided	19 (33.3)	10 (41.7)	42 (48.8)	39 (31.2)†0.010
pancolitis	29 (50.9)	12 (50.0)	30 (34.9)	64 (51.2)†0.019
backwash-ileitis	0	0	3 (3.5)	10 (8.0)*0.028
Extension:				
proctitis/ left-sided to pancolitis/ backwash	12/40 (30.0)	7/19 (36.8)	19/72 (26.4)	36/87(41.4)†0.048

*: $p \leq 0.05$ vs. smokers; #: $p \leq 0.05$ vs. quitters after diagnosis; †: $p \leq 0.05$ vs. quitters before diagnosis.
¶: Azathioprine, 6-mercaptopurine and/or methotrexate.
5-ASA: 5-aminosalicylates; EIM: extraintestinal manifestations; IQR: interquartile range; IS: immunosuppressants; PSC: primary sclerosing cholangitis.

(100% vs. 44%; p=0.038) and backwash-ileitis (16% vs. 4%; p=0.023) than non-passive smokers (n=75). No effect of passive smoking was observed on the need for medication, surgery, and hospitalizations (data not shown).

Multivariate analysis

In CD multivariate analysis with Cox-regression analysis identified only quitting smoking before diagnosis as a protective factor for at least one hospitalization (OR 0.64 (95%CI 0.43-0.95)). No independent factors were shown for at least two hospitalizations. For surgery, quitting smoking before diagnosis was not an independent factor whereas late onset of disease (OR 0.65 (95%CI 0.47-0.91)) and a continuous B1 disease behaviour during follow-up (OR 0.13 (95%CI 0.06-0.30)) were protective factors for at least one surgical procedure.

In UC multivariate analysis identified smoking after diagnosis as a protective factor for colectomy (OR 0.27 (95%CI 0.11-0.67)) whereas pancolitis at diagnosis (OR 3.18 (95%CI 1.85-5.48)) was a risk factor. Never smoking was a risk factor for the development of PSC in UC patients (OR 4.32 (95%CI 1.52-12.25)). Proctitis (OR 0.09 (95%CI 0.02-0.39)) and left-sided colitis at diagnosis (OR 0.35 (95%CI 0.13-0.93)) were associated with a lower risk for PSC.

Discussion

This study shows that active smoking has a dose-dependent beneficial effect on UC and smoking cessation after diagnosis was detrimental for UC patients. Remarkably, we did not observe a detrimental effect of active smoking on the disease course of CD, while our results suggest a detrimental role for passive smoking in CD. The differences in active smoking behaviour at diagnosis between CD patients, the general population and UC patients as observed by previous investigators were confirmed, with 52% smokers in CD, 41% in the general population and 28% in UC. Fortunately, many of the CD patients stop smoking after diagnosis, as the number of present smokers in CD is lower than in the general population (26% vs. 35%).

In contrast to the detrimental role of smoking on CD in previous studies, our study with a mean follow-up of 13 years showed that smokers had a later onset of disease than never smokers and also experienced fewer extraintestinal manifestations. The relation between never smoking and early disease onset has been shown before[32] and it advocates for a more prominent role of other predisposing factors in never smokers, in particular genetic predisposition. Quitters before diagnosis had less hospitalizations and surgery than smokers, but we think this is mainly caused by the longer follow-up of smokers. Studies from Israel,[18,19] Spain[15,16] and Norway[14] showed no detrimental effect of smoking on the course of CD either. In the studies from Spain and Israel the smoking behaviour of CD patients did not differ from a control group. The different effect of smoking on the disease course between our study and former studies that showed a detrimental effect of smoking,[6-12] could be explained by the longer follow-up period of our study (mean 13 years vs. 3-10 years). Furthermore, there are limitations to some studies that showed a detrimental effect of smoking. Studies showed that smoking was a risk factor for recurrence after surgery,[10,11,33] but this could not be confirmed in other post-operative recurrence studies.[13,17,20,34] Cosnes et al. showed in a

group of 400 CD patients that smokers needed more steroids and immunosuppressants,[8] but this was only true for women and there was no effect on the need for surgical intervention. In another study from Cosnes et al. the negative role of smoking was also only present in female CD patients.[26] We observed no detrimental effect of smoking in female CD patients. The follow-up of patients at our outpatient clinic is independent of complaints. It is therefore unlikely that our results are biased by patients who have withdrawn from follow-up because of dissolving symptoms after smoking cessation, and therefore were not included in our study. Our results are not biased by the drastic decline in active smokers between diagnosis and end of follow-up, because a subgroup analysis only showed a higher need for iv steroids in the continuous smokers. Early introduction of immunomodulators or biologicals in smoking CD patient could bias the results for hospitalizations and surgery.[8] However, only 57% of our patients were on immunomodulators and/or biologicals without any differences between smokers and never smokers.

Surprisingly, in contrast to active smoking, we found a detrimental effect of passive smoking in never smokers on the disease course of CD. Never smoking CD patients regularly exposed to cigarette smoke more often needed immunosuppressants and infliximab than rarely exposed patients, while their follow-up was much shorter. Since levels of nicotine metabolites are much lower in passive than in active smokers,[35,36] we think that other factors in an environment with tobacco smoke pollution than nicotine are involved. To confirm our findings, studies with levels of nicotine metabolites for defining passive smokers are needed.

We showed no effect of active smoking on the efficacy of the anti-TNF-α antibody infliximab in CD patients. Literature about infliximab and smoking is ambiguous. Some studies showed no effect of smoking on the response to infliximab,[37,38] but a smaller study showed that in 59 patients with luminal disease non-smokers responded better to infliximab than smokers (73% versus 22%; $P<0,001$).[39] Recently, there was also no effect of smoking on outcome in CD in a study comparing a top-down and a step-up strategy with infliximab.[40] Studies with the anti-TNF-α antibody certoluzimab also did not show an effect of smoking on efficacy (data on file union chimique belge).[41]

Our study did confirm the positive effect of smoking on UC. Even with a longer follow-up, smokers needed less often colectomy (12%) than never smokers (28%) with an OR of 0.27 (95% CI 0.11-0.67). This is in agreement with a meta-analysis (1,489 patients) showing an OR of 0.57 (95% CI 0.38-0.85) for a colectomy in current smokers compared to non-smoking UC patients.[23] Never smokers also had more backwash-ileitis than smokers. In addition, a clear dose-dependent effect of smoking was observed in UC. Heavy smoking UC patients needed fewer steroids, immunosuppressants and hospitalizations, and in heavy smokers the disease was more often limited to the rectum. Recently, it was shown that pack-years were associated with less extensive disease.[42] No beneficial effect of passive smoking could be established. In fact, passive smoking UC patients had more ileal disease (pouchitis and backwash-ileitis) than non-passive smoking UC patients. Further studies concerning the effect of passive smoking on UC are needed.

The differences between quitters before and after diagnosis of UC indicate that smoking cessation after diagnosis is detrimental for UC patients. Quitters after diagnosis needed more oral steroids and hospitalizations than quitters before diagnosis. Smoking is considered a protective environmental factor. Patients who quit smoking after diagnosis developed ulcerative colitis despite their smoking. Probably there are genetic or other environmental factors that make them more prone for developing ulcerative colitis. So when after the diag-

nosis another factor favouring activity of the colitis occurs, like smoking cessation, the disease course worsens. This may implicate that patients who experience worsening of disease after smoking cessation could benefit more from smoking than patients who quitted smoking before diagnosis.

PSC is an infamous extraintestinal disorder in IBD, particularly associated with UC. We showed in multivariate analysis an important association between PSC and non-smoking in UC (OR 4.32; 95% CI, 1.52-12.25). This is in accordance with other studies[43-45] and is also reported for PSC without underlying IBD.[45] Just like in UC, the protective mechanism of smoking on the development of PSC has not yet been clarified. Probably there is a similar mechanism.

It is important to note the putative limitations of our study. The retrospective design may have led to bias in the interpretations of the data. Asking about smoking behaviour in the past and the influence of the diagnosis of the disease on smoking behaviour, could introduce a recall bias. This was counteracted by comparing answers from the questionnaire with data from the medical charts, but information about passive smoking was not available from the charts. Using a survey may lead to selection bias, but the high response-rate to our questionnaire (82%) makes it highly likely that this study is well representative of the smoking behaviour in our IBD population. So it is unlikely that our results are biased by using a questionnaire, especially for active smoking.

In conclusion, no effect of active smoking was shown on the course of CD, but passive smoking was detrimental for CD patients. In UC, active smoking had dose-dependent beneficial effects on the course of the disease, while passive smoking seems detrimental for the ileum of these patients. The differences in active smoking behaviour at diagnosis between CD patients, the general population and UC patients were confirmed, but the number of present smokers in CD is lower than in the general population. Passive smoking is a novel risk factor for IBD.

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Chapter 4

Effects of active and passive smoking on Crohn's disease and ulcerative colitis in a cohort from a regional hospital

F. van der Heide, M.Wassenaar, K. van der Linde, P. Spoelstra, J.H. Kleibeuker, G. Dijkstra

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Abstract

Introduction:
Smoking is detrimental for Crohn's disease (CD), but beneficial for ulcerative colitis (UC). Previously we studied the effects of active and passive smoking in CD and UC patients from a university hospital. The present study was conducted to assess the same effects in patients from a regional hospital.

Methods:
A questionnaire focussing on cigarette smoke exposure was sent to 382 patients. Returned questionnaires (84%: 128 CD and 192 UC patients) were incorporated into a retrospective chart review about disease behaviour and received therapy.

Results:
At diagnosis there were 52% (95% confidence interval (CI): 43%-60%) smokers among CD patients, 40% in a control population and 25% (95% CI: 18%-31%) among UC patients. There were less former (19% vs. 31%; p=0.013) and never smokers at diagnosis (30% vs. 44%; p=0.009) in CD than in UC. No detrimental effects of active or passive smoking on the course of CD were observed. UC patients who continued smoking after diagnosis needed less often two or more hospitalizations than never smokers (5% vs. 25%; p=0.036). Otherwise no clear beneficial effects of active smoking on UC were observed. Passively smoking UC patients experienced more often extraintestinal manifestations (25% vs. 7%; p=0,029) than non-passive smokers.

Conclusion:
Also in a regional hospital inflammatory bowel disease population smoking is a risk factor to develop CD and protects against developing UC. We found no detrimental effects of smoking on the course of CD and no clear beneficial effects on the course of UC.

Introduction

Smoking is a well established environmental factor in inflammatory bowel disease (IBD) with remarkable opposite effects on Crohn's disease (CD) and ulcerative colitis (UC). Smoking is a risk factor for developing CD, but it protects against developing UC.[1,2] In addition, smoking is also unfavourable for the disease course of CD with a higher need for steroids, azathioprine and surgery in smokers than in non smokers.[3-9] In contrast, smoking is beneficial for the disease course of UC with a lower need for hospitalizations, steroids and colectomy in smokers than in non smokers.[9-15] Recently, we studied the effects of smoking in a Dutch IBD population from a university hospital.[16] We confirmed the effects of active smoking on the development of CD and UC, with 52% smokers in CD, 41% in the control population and 28% in UC. We also confirmed the beneficial effect of active smoking in UC and moreover showed that this beneficial effect was dependent on the daily cigarette dose. However, we could not confirm the unfavourable effects of active smoking on the disease course of CD.

In addition to the effects of active smoking, we also studied the effects of passive smoking on the disease course in our previous study. These were not in line with the effects of active smoking. First, our data suggested a detrimental effect of passive smoking on the disease course of CD while active smoking had no effect. Never smoking CD patients regularly exposed to cigarette smoke more often needed immunosuppressants and infliximab than rarely exposed patients. Second, in UC no beneficial effect of passive smoking on the disease course was found while active smoking was beneficial. In fact, passive smoking UC patients had more ileal disease (pouchitis and backwash-ileitis) than non-passive smoking UC patients.

So the results of our previous study on the effects of active and passive smoking on IBD were somewhat unexpected, mainly because the detrimental effect of active smoking on CD was not confirmed, but also because of the finding of opposite effects of active and passive smoking. This could be caused by having performed our study in a university hospital with an important referral function for the northern part of the Netherlands. Studying patients referred from other hospitals causes a selection bias, since CD patients with a more benign disease course are underrepresented.[17] The aim of the present study was to see whether our previous findings could be confirmed in an IBD cohort from a regional hospital. For this purpose, we analyzed the relationship between active and passive smoking, and the course and behaviour of CD and UC over a mean time frame of 12 years in an IBD cohort from a regional hospital.

Methods

Patients

Three-hundred and eighty-two patients who visited the outpatient department of the Medical Center Leeuwarden in Leeuwarden, the Netherlands, in 2006 and 2007, and who had a confirmed diagnosis of CD or UC by endoscopic, radiologic and/or pathologic examinations[18] were asked to participate in this study. Patients are seen at a regular basis and more often, if needed, at the outpatient department. The Medical Center Leeuwarden is a regional hospital and the largest in the province of Friesland. Clinical characteristics and the outcome

variables were obtained through retrospective analysis of the medical charts. In addition to the information obtained from the charts, detailed information about smoking behaviour was obtained through a written questionnaire, which was sent to all 382 patients. Three-hundred and twenty patients responded to the questionnaire (84%), with 128 CD and 192 UC patients, and these 320 constituted the final study population. Smoking behaviour at diagnosis (median year of diagnosis 1996) of CD and UC patients were compared with 1996-records of a general, age adjusted Dutch population of the StatLine databank of the Dutch central agency for statistics (Statistics Netherlands; http://statline.cbs.nl). The methods used were discussed with the medical ethics committee. According to Dutch legislation there were no objections against the methods used. A returned questionnaire was considered as an informed consent.

Definitions of smoking behaviour

For defining smoking behaviour, all patients received a detailed questionnaire about smoking behaviour. This included questions about 1) whether the patient had ever been smoking and, if so, number of years smoked, and, if a former smoker, number of months after cessation; 2) product smoked and average amount smoked per day; 3) the relation between diagnosis and smoking behaviour; 4) passive smoking at present (every day, a few times a week, once a week, or never/seldom more than 1 hour a day in the same room with smoking persons); 5) educational level; and 6) family history of IBD.

At the time of diagnosis patients were categorized as smokers, former smokers and never smokers. A smoker at diagnosis started smoking seven or more cigarettes per week at least six months before diagnosis, a former smoker quitted smoking at least six months before diagnosis and a never smoker had never smoked until six months before diagnosis.

For studying the relation between smoking and disease course, patients were divided into three groups: 1) smokers after diagnosis (smoker at diagnosis who did not stop smoking within one year after diagnosis, or former smoker at diagnosis who restarted smoking within one year after diagnosis, or never smoker at diagnosis who started smoking within one year after diagnosis), 2) former smokers (former smoker at diagnosis who did not restart smoking within one year after diagnosis, or smoker at diagnosis who stopped smoking within one year after diagnosis) and 3) never smokers after diagnosis (never smoked one year after diagnosis).

The level of smoke exposure was analyzed by dividing smokers after diagnosis into two groups according to the median number of cigarettes smoked per day. Smoking of one cigar was considered equal to four cigarettes, as the average cigar contains four grams of tobacco and a cigarette one gram.[19] For studying the relation between passive smoking and disease course, never smokers after diagnosis were divided into two groups: 1) passive smokers (daily, few times a week or once a week exposed to a smoking environment) and 2) non-passive smokers (rarely or never exposed).

Clinical characteristics and outcome variables

The patient characteristics and outcome variables we recorded were described before.[16] Shortly, for CD we used the Vienna-classification for describing age at diagnosis (early onset <40 years and late onset >40 years), and disease behaviour and location.[20] For UC the location was defined as proctitis, left-sided colitis and pancolitis with or without backwash-ileitis, and determined at diagnosis and as the maximal extend during follow-up. We recorded

hospitalizations, surgical procedures, extraintestinal manifestations (EIM), including primary sclerosing cholangitis, and the use of 5-aminosalicylates (5-ASA), steroids, azathioprine, 6-mercaptopurine, methotrexate, cyclosporine and infliximab at any time during follow-up. All events were recorded till the end of follow-up (July 2007), or the last date of clinical or outpatient visit for patients who were discharged or had withdrawn from outpatient control. For UC patients after a colectomy and without development of pouchitis, the date of colectomy was the end of follow-up.

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences program version 14.0 (SPSS Inc., Chicago, Illinois, USA). Descriptive variables are presented as medians (interquartile range (IQR)) and categorical variables as frequencies with percentages. Frequencies were compared with the chi-square test and means between two groups with the Mann-Whitney test. Differences are considered significant when $p \leq 0.05$. The odds ratios (OR) and the 95% confidence intervals (CI) were calculated if appropriate.

Results

Patient characteristics and smoking behaviour

Characteristics of the 320 respondents are presented in table 1. CD patients were more often female and younger at diagnosis, and more often experienced EIM than UC patients. At diagnosis CD patients were more often smokers while UC patients were more often former and never smokers. There were 52% (95% CI: 43%-60%) smokers in CD and 25% (95% CI: 18%-31%) in UC. At the median time point of diagnosis (1996) 40% of the control population were smokers. This means an OR of 1.29 (95% CI: 1.07-1.51) for CD and an OR of 0.61 (95% CI: 0.46-0.77) for UC for being a smoker at diagnosis.

Patient characteristics according to smoking behaviour at diagnosis

In CD, former smokers at diagnosis were older at that time than smokers (38.0 (IQR 27.7-52.3) vs. 29.7 (22.9-41.7) years; $p=0.023$) and never smokers (38.0 vs. 26.2 (IQR 19.3-41.2) years; $p=0.008$). In UC, there were more females among never smokers at diagnosis than among smokers (57% vs. 28%; $p=0.001$) and former smokers (57% vs. 38%; $p=0.031$). Former smokers at diagnosis had less often an early onset of disease than smokers (43% vs. 66%; $p=0.020$) and never smokers (43% vs. 71%; $p=0.001$). Never smokers were the youngest at diagnosis, followed by smokers, whereas former smokers were the oldest at diagnosis (30.0 (IQR 22.6-43.6) vs. 34.7 (31.4-47.6) vs. 41.3 (33.8-54.6) years; $p<0.04$). Smokers had more often completed only lower education (35% vs. 19%; $p=0.046$) and less often medium education (30% vs. 51%; $p=0.023$) than never smokers.

Smokers presented more often with a pancolitis at diagnosis than never smokers (51% vs. 30%; $p=0.021$). Since in UC smokers and never smokers differed in gender, we looked at differences in pancolitis at diagnosis between females and males. Females presented less often with pancolitis at diagnosis than males (28% vs. 45%; $p=0.017$). Multivariate analysis with logistic regression revealed that gender was an independent risk factor for pancolitis at diagnosis (OR for females: 0.52; 95% CI: 0.27-1.00), but smoking behaviour at diagnosis was not.

Table 1. Characteristics of the Crohn's disease and ulcerative colitis patients responding to the questionnaire

Characteristic: n (%)	CD (n=128)	UC (n=192)	p-value
Gender: female	79 (61.7)	84 (43.8)	0.002
Age at diagnosis:			
median (yrs) (IQR)	30.1 (22.8-41.8)	35.2 (27.2-49.6)	0.001
early onset: n (%)	90 (70.3)	117 (60.9)	ns
Follow-up: median (yrs) (IQR)	10.4 (3.8-20.5)	8.3 (3.5-17.1)	ns
Education:			
lower	29 (22.7)	50/190 (26.3)	ns
medium	62 (48.4)	79 (41.6)	ns
higher	37 (28.9)	61 (32.1)	ns
Family history of IBD	29/116 (25.0)	41/170 (24.1)	ns
Smoking behaviour at diagnosis:			
smoker	66 (51.6)	47 (24.5)	<0.001
former smoker	24 (18.8)	60 (31.3)	0.013
never smoker	38 (29.7)	85 (44.3)	0.009
EIM	31 (24.2)	17 (8.9)	<0.001
Primary sclerosing cholangitis	1 (0.8)	6 (3.1)	ns
Location CD:			
terminal ileum	40 (31.2)		
colon	45 (35.2)		
ilcocolon	37 (28.9)		
upper GI-tract	6 (4.7)		
Behaviour CD*:			
B1	57 (44.5)		
B2	26 (20.3)		
B3	45 (35.2)		
Location at diagnosis UC:			
proctitis		49/176 (27.8)	
left-sided		61 (34.7)	
pancolitis		66 (37.5)	
backwash-ileitis		0	
Maximal location UC:			
proctitis		15/190 (7.9)	
left-sided		58 (30.5)	
pancolitis		117 (61.6)	
backwash-ileitis		0	

*: B1: non-stricturing, non-penetrating; B2: stricturing; B3: penetrating.
CD: Crohn's disease; EIM: extraintestinal manifestations; IQR: interquartile range; UC: ulcerative colitis.

Disease outcome in CD

Active smoking

The relation between smoking behaviour after diagnosis and disease outcome in CD is shown in table 2. There were no differences between smokers and never smokers after diagnosis for the outcome variables, except that smokers after diagnosis had less often stricturing disease behaviour than never smokers. Smokers needed less often one or more hospitalizations and received less often iv steroids than former smokers.

Thus no main differences between the outcome of smokers and never smokers were observed. It is possible that these findings are biased by our definition of smokers after diagnosis, that is, no smoking cessation within one year after diagnosis. Following this definition a patient that stopped smoking two years after diagnosis is called a smoker after diagnosis. Some of our so-called smokers after diagnosis probably have stopped smoking more than a year after diagnosis, since there is a drastic decline in active smokers between time of diagnosis and end of follow-up (from 52% to 28%). Therefore, we composed a subgroup of patients that smoked during the complete follow-up and compared them with the never smokers at the end of follow-up. This analysis showed no differences for the outcome variables, except that the subgroup of smokers had more often localisation of the disease proximal to the terminal ileum than never smokers (14% vs. 0%; p=0.022). We did not observe differences in outcome between smokers and never smokers among female CD patients either.

We also studied the effects of number of cigarettes smoked per day on the disease course in smokers after diagnosis. This analysis showed no differences for the outcome variables, except that CD patients smoking ten or less cigarettes per day (n=30) needed more often one or more hospitalizations (80% vs. 50%; p=0.018) and had less often non-stricturing, non-penetrating disease behaviour (30% vs. 61%; p=0.019) than patients smoking more than ten cigarettes per day (n=28).

Passive smoking

For studying the effects of passive smoking on the disease course of CD, never smoking patients were divided in passive (n=14) and non-passive smokers (n=22). This analysis showed no differences for the outcome variables.

Disease outcome in UC

Active smoking

The relation between smoking behaviour after diagnosis and disease outcome in UC is shown in table 3. No differences were observed for the outcome variables. Smokers after diagnosis more often had pancolitis at diagnosis, and subsequently as maximal localization during follow-up, than former and never smokers. Thus, we did not observe a beneficial effect of active smoking on UC. As suggested above in CD, it is possible that these findings are biased by our definition of smokers after diagnosis, since there is also a drastic decline in active smokers between time of diagnosis and end of follow-up in UC (from 25% to 13%). Therefore, we composed a subgroup of patients that smoked during the complete follow-up and compared them with the never smokers at the end of follow-up. This subgroup of continuing smokers used less often topical 5-ASA (64% vs. 87%; p=0.010) and needed less often two or more hospitalizations (5% vs. 25%; p=0.036) than never smokers.

We also studied the effects of number of cigarettes smoked per day on the disease course in smokers after diagnosis. No differences were observed on the outcome variables between

Table 2. Outcome variables for Crohn's disease according to smoking behaviour after diagnosis

Characteristic (%)	Smokers (n=59)	Former smokers (n=32)	Never smokers (n=37)
Follow-up: median (yrs) (IQR)	11.4 (4.8-21.2)	9.6 (3.3-24.4)	9.9 (2.4-19.7)
Hospitalizations:			
at least one	37/57 (64.9)	28/30 (93.3)*0.004	27/35 (77.1)
at least two	27/55 (49.1)	14/30 (46.7)	17/34 (50.0)
Surgery:			
at least one	25 (42.4)	14 (43.8)	21 (56.8)
at least two	11 (18.6)	9 (28.1)	11 (29.7)
No therapy	0	1 (3.1)	0
5-ASA:	52 (88.1)	24/30 (80.0)	32 (86.5)
topical	16/57 (27.1)	10/27 (37.0)	9 (24.3)
oral	52 (88.1)	23/29 (79.3)	31 (83.8)
Steroids:	46/58 (79.3)	25/30 (83.3)	31/36 (86.1)
topical	4/56 (7.1)	5/29 (17.2)	3/36 (8.3)
oral	46/58 (79.3)	25/30 (83.3)	30/35 (85.7)
iv	8/54 (14.8)	9/25 (36.0)*0.033	6/32 (18.8)
IS¶	28 (47.5)	19 (59.4)	16 (43.2)
Infliximab	3 (5.1)	2 (6.3)	3 (8.1)
EIM	13 (22.0)	6 (18.8)	12 (32.4)
Primary sclerosing cholangitis	0	0	1 (2.7)
Location:			
ileum	15 (25.4)	11 (34.4)	14 (37.8)
colon	19 (32.2)	13 (40.6)	13 (35.1)
ileocolon	20 (33.9)	7 (21.9)	10 (27.0)
upper GI-tract	5 (8.5)	1 (3.1)	0
Behaviour§:			
B1	27 (45.8)	14 (43.8)	16 (43.2)
B2	7 (11.9)	8 (25.0)	11 (29.7)*0.029
B3	25 (42.4)	10 (31.3)	10 (27.0)

*: $p \leq 0.05$ vs smokers. ¶: Azathioprine, 6-mercaptopurine and/or methotrexate. §: B1: non-stricturing, non-penetrating; B2: stricturing; B3: penetrating.
5-ASA: 5-aminosalicylates; EIM: extraintestinal manifestations; IQR: interquartile range; IS: immunosuppressants.

patients smoking twelve or less cigarettes per day (n=20) and patients smoking more than twelve cigarettes per day (n=19).

Passive smoking

Never smoking UC patients were divided in passive and non-passive smokers. Passive smokers (n=24) experienced more often EIM (25% vs. 7%; p=0,029) than non-passive smokers (n=55). No differences were observed for the other outcome variables.

Table 3. Outcome variables for ulcerative colitis according to smoking behaviour after diagnosis

Characteristic (%)	Smokers (n=39)	Former smokers (n=69)	Never smokers (n=84)
Follow-up: median (yrs) (IQR)	9.2 (4.0-19.2)	8.2 (3.6-12.4)	8.2 (3.2-17.6)
Hospitalizations:			
at least one	16/38 (42.1)	27/67 (40.3)	39/79 (49.4)
at least two	6/38 (15.8)	16/67 (23.9)	19/79 (24.1)
Colectomy	3 (7.7)	13 (18.8)	8 (9.5)
Pouch	0	5 (7.2)	5 (6.0)
Pouchitis	0	4/5 (80.0)	3/5 (60.0)
No therapy	0	0	0
5-ASA:	39 (100)	68 (98.6)	83/83 (100)
topical	29/38 (76.3)	58/67 (86.6)	71/82 (86.6)
oral	38 (97.4)	60 (87.0)	75/83 (90.4)
Steroids:	24/37 (64.9)	42 (60.9)	54/83 (65.1)
topical	13/38 (34.2)	22/68 (32.4)	17/80 (21.3)
oral	22/37 (59.5)	38 (55.1)	50/83 (60.2)
iv	3/33 (9.1)	11/66 (16.7)	11/79 (13.9)
IS¶	8 (20.5)	19 (27.5)	28 (33.3)
Cyclosporine	2 (5.1)	1 (1.4)	2 (2.4)
EIM	2 (5.1)	5 (7.2)	10 (11.9)
Primary sclerosing cholangitis	1 (2.6)	1 (1.4)	4 (4.8)
Location at diagnosis:			
proctitis	8/36 (22.2)	18/67 (26.9)	23/73 (31.5)
left-sided	7 (19.4)	25 (37.3)	29 (39.7)*0.034
pancolitis	21 (58.3)	24 (35.8)*0.028	21 (28.8)*0.003
backwash-ileitis	0	0	0
Maximal location:			
proctitis	1 (2.6)	5 (7.2)	9/82 (11.0)
left-sided	8 (20.5)	25 (36.2)	25 (30.5)
pancolitis	30 (76.9)	39 (56.5)*0.034	48 (58.5)*0.048
backwash-ileitis	0	0	0
Extension: proctitis/ left-sided to pancolitis/ backwash	8/15 (53.3)	12/43 (27.9)	22/52 (42.3)

*: $p \leq 0.05$ vs. smokers. ¶: Azathioprine, 6-mercaptopurine and/or methotrexate.
5-ASA: 5-aminosalicylates; EIM: extraintestinal manifestations; IQR: interquartile range; IS: immunosuppressants.

Comparison of patients from the regional hospital with patients from a tertiary referral hospital

Finally, we compared the disease course between patients from the present study and patients from our previous study.[16] In CD, we confirmed that patients from a regional hospital have a more benign disease course; they needed less often one or more surgical procedures (47% vs. 58%; p=0.023) and infliximab (6% vs. 19%; p=0.001), and had more often

localisation of the disease only in the colon (35% vs. 26%; p=0.042) and non-stricturing, non-penetrating disease behaviour (45% vs. 34%; p=0.027) than patients from the university hospital (n=380). On the other hand, patients from the regional hospital had more often iv administration of steroids (21% vs. 10%; p=0.002).

In UC, we also found a more benign disease course in patients from the regional hospital; they needed less often a colectomy (13% vs. 20%; p=0.025) and topical steroids (28% vs. 68%; p<0.001) than patients from the university hospital (n=295). An exception on this was that patients from the regional hospital more often had pancolitis as maximal localization during follow-up (62% vs. 46%; p<0.001).

Discussion

Smoking is a remarkable risk factor for CD and UC, the two most common IBDs. Smoking is detrimental for the development and course of CD, while smoking is beneficial for the development and course of UC. In our previous study in an IBD population from a Dutch university hospital we confirmed these effects of smoking on the development of both CD and UC, and on the disease course of UC.[16] However, we did not observe a detrimental effect of active smoking on the course of CD. The present study was conducted to see whether our findings could be confirmed in an IBD population from a regional hospital. We indeed confirmed that in a regional hospital population smoking is a risk factor for developing CD and protects against developing UC, with more active smokers at diagnosis in CD, and more former and never smokers in UC. And as in the university hospital population, we did not observe a detrimental effect of active smoking on the disease outcome in CD. However, we did not confirm that active smoking was beneficial for the course of UC, although in a subgroup analysis continuing smokers needed less often two or more hospitalizations than never smokers.

So we confirmed in this IBD population from a regional hospital that smoking is a risk factor for developing CD and protective for UC. At diagnosis there were 52% smokers in CD, 40% in the control population and 25% in UC. These numbers are comparable with those from our university population with 52% smokers in CD and 28% in UC.[16] In a recent meta-analysis of 1,679 CD and 2,459 UC patients active smokers had an OR of 1.76 (95% CI: 1.40-2.22) for developing CD and an OR of 0.58 (95% CI: 0.45-0.75) for developing UC.[2] Former smokers had an increased risk for UC (OR 1.79; 95% CI: 1.37-2.34). More than half of the patients in this meta-analysis were from regional hospital IBD populations.

In contrast to most reports so far, we neither observed a detrimental effect of smoking on the disease course of CD in a regional nor in a university hospital population from the Netherlands. In fact, in the present study smokers needed less often a hospitalization and iv steroids than former smokers, and heavy smoking CD patients needed less often a hospitalization and had more often non-stricturing, non-penetrating disease behaviour than light smokers. Other studies could also not confirm the detrimental effect of smoking on CD.[11-13,15,21-24] Of these studies, two were performed in regional hospital populations.[11,15] Of the studies showing a detrimental effect only a few were performed in regional hospital populations.[3,7] The question remains why we do not observe a detrimental effect of smoking in our CD patients from a regional and a university hospital. The length of follow-up in our regional hospital

population (mean 13.5 years) is comparable with that of our university hospital population (mean 13 years) and is longer compared to that in studies that showed a detrimental effect of smoking (between 3 and 10 years).[3-8] A possible bias, especially for the results on hospitalizations and surgery, could be early introduction of immunosuppressants or biologicals in smoking CD patients.[5] However, only 50% of our patients were on immunosuppressants and/or biologicals without any differences between smokers and never smokers.

We did not observe a clear positive effect of smoking on the course of UC patients from this regional hospital population, although from a subgroup of continuing smokers after diagnosis fewer needed two or more hospitalizations than never smokers. In our study in the university hospital population we found a lower colectomy rate in smokers than in never smokers (12% vs. 28%),[16] and a meta-analysis (1,489 patients) showed an OR of 0.57 (95% CI: 0.38-0.85) for a colectomy in current smokers compared to non-smoking UC patients.[9] The fact that we did not observe a beneficial effect of smoking on colectomy rate in the regional hospital population could be caused by a lack of power considering the low number of smokers (39) and a lower colectomy rate in the regional than in the university hospital population (13% vs. 20%).

In the present study we could not confirm our findings in our university hospital population on the role of passive smoking in both CD and UC.[16] In that population our data suggested a detrimental effect of passive smoking on both CD (passive smokers needed more often immunosuppressants and infliximab) and UC (passive smokers had more ileal disease (pouchitis and backwash-ileitis)). Both findings were not in line with the effects of active smoking in that study (no effect of active smoking in CD and beneficial effect of active smoking in UC). In the present study, we found no effect of passive smoking in CD. In UC, passive smokers more often experienced EIM, but we found no differences for ileal disease. However, the latter was not surprising, since pouchitis and backwash-ileitis were seldom seen in our regional hospital population. Considering our inconsistent findings regarding the effects of passive smoking in itself and compared to active smoking, studies about the effects of passive smoking have to be repeated, ideally with measurements of biochemical markers of passive smoking such as urinary cotinine levels.

The present study has a number of limitations. The retrospective design may have led to bias in the interpretation of the data. A retrospective design could also lead to bias by including only patients who require follow-up. However, it is unlikely that our results are biased by patients who have withdrawn from follow-up because of resolving symptoms, since the follow-up of patients at the outpatient clinic is at a regular basis and more often, if needed. Using a questionnaire on smoking behaviour in the past could introduce a recall bias. Using a survey may lead to selection bias, but the high response rate to our questionnaire (84%) makes it highly likely that this study is well representative of the smoking behaviour in this IBD population. Another limitation is that patients were divided into three groups (smokers after diagnosis, former and never smokers) instead of the four groups (smokers after diagnosis, quitters before and after diagnosis and never smokers) in our previous study in the university hospital population. We chose for this division in three groups because otherwise groups became too small. However, the definitions of the smokers after diagnosis and the never smokers after diagnosis are the same in both studies, making comparisons between both studies still possible. Finally, the numbers of patients studied in the present study are lower than in our previous study, which could contribute to unexpected and/or non significant results.

In conclusion, we confirmed that in both a regional and university hospital population

smoking is a risk factor for CD and protective against the development of UC. In contrast to most reports, we neither observed a detrimental effect of active smoking on the disease course of CD in a regional nor a university hospital population. For UC, we found no clear beneficial effect of smoking on the disease course in this regional hospital population. For passive smoking, we could not confirm that passive smoking is detrimental for CD and/or for ileal disease in UC, as suggested in our university hospital population. The role of passive smoking in IBD needs further exploration.

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Chapter 5

Differences in genetic background between active smokers, passive smokers and non-smokers with Crohn's disease

F. van der Heide, I.M. Nolte, J.H. Kleibeuker, C. Wijmenga, G. Dijkstra, R.K. Weersma

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Abstract

Introduction:
Smoking behaviour and genetic variations are important factors for the development of Crohn's disease (CD), but studies investigating the interaction between smoking and genetic background are scarce. We studied allelic associations of 19 confirmed variants located in 14 CD-associated genes or loci, in CD patients stratified for active smoking at diagnosis and passive smoking in childhood.

Methods:
Genotyping data of 19 CD-associated single nucleotide polymorphisms (SNPs) were available for 310 CD patients and 976 controls. Data on active smoking at diagnosis and passive smoking in childhood were obtained through a written questionnaire and review of medical charts.

Results:
Loci associated in smoking, but not in non-smoking, CD patients were 5p13.1 (rs17234657), *DLG5* (rs2165047), *NKX2-3* (rs10883365) and *NOD2* (R702W). Loci associated in non-smoking, but not in smoking, CD patients were *IL23R* (rs7517847), 5p13.1 (rs9292777), *IRGM* (rs13361189 and rs4958847), *IL12B* (rs6887695) and *CCNY* (rs3936503). *PTPN2* (rs2542151) was only associated in the smoking CD cohort ($P=0.041$), and not in the entire cohort ($P=0.23$) and the non-smoking CD cohort ($P=0.80$). In passively smoking CD patients associations with 13 SNPs in 9 loci were found, including *PTPN2*. In non-passively smoking CD patients only associations with *NOD2* (1007fsinsC and G908R) were found.

Conclusion:
The difference in associated genes between smoking and non-smoking CD patients implies a complex gene-environment interaction. Therefore, genetic studies in CD should be stratified for smoking behaviour, as otherwise moderately associated genes like *PTPN2* can be missed.

Introduction

Crohn's disease (CD) is an inflammatory bowel disease (IBD) characterized by chronic relapsing inflammation of the gastrointestinal tract. Development of CD is influenced by both environmental factors and the genetic background. One of the most important environmental risk factors is smoking. Several studies have shown that CD patients are more likely to be smokers than their matched controls.[1-4] In addition, CD patients are more often exposed to tobacco smoke in childhood than controls.[5,6] So active smoking, and possibly also passive smoking, seems to be an important risk factor for the development of CD.

Development of CD is also influenced by the genetic background and the role of genetic variants has been the subject of intensive research the last decade. The first identified CD associated gene was *CARD15* on chromosome 16 encoding for the protein NOD2.[7,8] After the introduction of genome-wide association studies, there has been a enormous progress in the unravelling of the genetic background of CD, with more than 30 confirmed susceptibility loci to date.[9]

Although it is likely that development of CD is partly caused by an interaction between environmental factors such as smoking, and the genetic background, studies on the interaction between smoking and genetic variants are scarce. In a Spanish CD population of 178 patients the prevalence of the three CD-related mutations in the *NOD2* gene (R702W, G908R and 1007fsinsC) in smokers and non-smokers were studied and only the 1007fsinsC mutation was more frequent in non-smokers.[10] Two other studies of 338 and 232 CD patients showed that *NOD2* variants and smoking behaviour were independent risk factors for CD, but there was no specific interaction between them.[11,12] In a Hungarian CD population of 527 patients no difference was found in the proportion of smokers between carriers and non-carriers of *NOD2* variant alleles.[13] Finally, smoking status was combined with *TUCAN*, IBD5, *NOD1-2* and *TNFSF15* genotypes in a diagnostic panel to generate a risk scoring system for CD, but the interaction between smoking and the genetic variants was not studied.[14]

The aim of our study was to explore differences in genetic variants in relation to smoking status. For this purpose we used data available from previous studies [4,15-17] and selected three *NOD2* variants and 16 confirmed CD associated single nucleotide polymorphisms (SNPs), and studied the allelic associations in CD patients stratified for smoking status at diagnosis and for passive smoking in childhood.

Methods

Subjects

DNA samples were available for 310 CD patients from the University Medical Center Groningen, the Netherlands. Diagnosis of CD was based on accepted clinical, radiologic, endoscopic and histopathological criteria.[18] The controls consisted of 976 healthy volunteers recruited from the University Medical Center Utrecht, the Netherlands, and have been previously described.[15] All participants were of European Caucasian descent and gave informed consent. The study was approved by the ethics review committees of the participating hospitals. All DNA samples and data in this study were handled anonymously.

Patient characteristics recorded were gender, age at diagnosis (divided in early age onset

(<40 years) and late onset disease (>40 years) according to Vienna-classification),[19] time of diagnosis (defined as the date of the first detection of inflammatory abnormalities by radiological, endoscopic or peroperative examinations), disease behaviour and location according to Montreal-classification,[20] and extraintestinal manifestations.

Genotyping and SNP selection

Genotype data was available from previous studies and genotyping methods have been described before.[15-17] For the current study we included 19 SNPs located in 14 confirmed CD susceptibility loci (table 2).

Data on smoking behaviour

Information on active smoking behaviour at diagnosis was obtained from medical charts and from a written questionnaire that we described before.[4] Patients were divided in smokers and non-smokers at diagnosis. Smokers were defined as having started smoking seven or more cigarettes per week at least six months before diagnosis. Non-smokers were defined as never smokers at diagnosis and smokers who quitted smoking six or more months before diagnosis. In five of 310 CD patients it was not possible to define smoking behaviour at diagnosis. Smoking behaviour at diagnosis (median year of diagnosis was 1995) of CD patients was compared with 1995-records of a general, age adjusted Dutch population of the StatLine databank of the Dutch central agency for statistics (Statistics Netherlands; http://statline.cbs.nl), as described before.[4]

Information on passive smoking in childhood was obtained from the same written questionnaire, which also included questions about passive exposure to cigarette smoke in childhood. Patients were asked whether they were exposed to cigarette smoke at home being a child. With this information patients were divided in passive smokers (exposed to cigarette smoke) and non-passive smokers (not exposed to cigarette smoke). For 225 of the 310 CD patients information about passive smoking in childhood was available.

Statistical analysis

For patient characteristics, continuous variables are presented using medians (range) and categorical ones using frequencies with percentages. Data were analyzed using the Statistical Package for the Social Sciences program version 12.0 (SPSS Inc., Chicago, Illinois, USA). Continuous variables were compared with the Mann-Whitney test and categorical ones with the chi-square test. Differences in allele distribution between cases and controls, and within cases were tested for significance by the chi-square test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using Woolf's method with Haldane's correction. The significance threshold for *P* values was set at <0.05.

Results

Patient characteristics

The characteristics of all 310 CD patients are listed in table 1. Most patients were female, had an early onset of disease and a history of surgical intervention. The percentage of patients that were smokers at diagnosis (54% (95%CI: 48.1%-59.4%) is higher than the 41%

Table 1. Characteristics of 310 CD patients

Characteristic: n (%)	
Gender: female	203 (65.5)
Age at diagnosis (years):	
median (range)	26.6 (7.5-73.9)
early onset (<40 years)	234 (75.5)
Follow-up (years) median (range)	11.0 (1.4-51.4)
Smoking at diagnosis	164/305 (53.8)
Passive smoking in childhood	160/225 (69.0)
Disease localization:	
ileal	88 (28.4)
colon	72 (23.2)
ileocolon	150 (48.4)
Upper GI tract	19 (6.1)
Disease behaviour:	
non-stricturing, non-penetrating	104 (33.5)
stricturing	75 (24.2)
penetrating	131 (42.3)
Peri-anal disease	88 (28.4)
Extraintestinal manifestations	55 (17.7)
Family history of IBD	90 (29.0)
History of surgical intervention	206 (66.5)

CD - Crohn's disease; GI - gastrointestinal; IBD - inflammatory bowel disease.

smokers in the general population at the median time point of diagnosis (1995).[4]

Clinical characteristics of smokers were compared to those of non-smokers. Smokers were older at diagnosis than non-smokers (median 29.2 vs 25.1 years; $P=0.021$) and smokers were more often exposed to cigarette smoke in childhood than non-smokers (77.2% vs 63.4%; $P=0.023$). No differences for the other clinical characteristics listed in table 1 were observed. In addition, at diagnosis we looked at a combined effect of *NOD2* status and smoking behaviour on disease location and behaviour. No differences were found for patients with any *NOD2* variant divided in smokers and non-smokers, and for smoking patients divided in patients with no *NOD2* variants and with any *NOD2* variant on this matter.

Allelic association analysis stratified for smoking behaviour at diagnosis

The results of the allelic association analysis between the entire cohort of 310 CD patients and 976 healthy controls are shown in table 2 and supplementary table 1. Fourteen SNPs located in 10 loci were statistically significant associated with CD in this cohort. We then analyzed whether the genetic associations were different between the cohorts of smoking and non-smoking CD patients. The results of the allelic association analysis stratified for smoking behaviour at diagnosis are also depicted in table 2. In both smoking and non-smoking CD patients allelic associations with *IL23R* (rs11209026) and *NOD2* (1007fsinsC and G908R) were found. In smoking, but not in non-smoking CD patients, associations with 5p13.1 (rs17234657), *DLG5* (rs2165047), *NKX2-3* (rs10883365) and *NOD2* (R702W) were found.

Table 2. Allelic association for 19 CD associated SNPs for all CD patients (n=310), smoking CD patients (n=164) and non-smoking CD patients (n=141) compared to controls (n=976)

rs number	Chr	Locus	All patients vs controls (*P* value)	Smokers vs controls (*P* value)	OR smokers vs controls (95% CI)	Non-smokers vs controls (*P* value)	OR non-smokers vs controls (95% CI)
rs11209026	1	IL23R	**4.45E-07**	**4.03E-04**	**0.22 (0.09-0.55)**	**2.59E-04**	**0.15 (0.05-0.49)**
rs7517847	1	IL23R	**0.0060**	0.052	0.77 (0.60-1.00)	**0.034**	**0.74 (0.56-0.98)**
rs12035082	1	1q24	**0.044**	0.11	1.23 (0.95-1.60)	0.16	1.21 (0.92-1.59)
rs2241880	2	ATG16L1	**0.046**	0.053	0.78 (0.61-1.00)	0.29	0.86 (0.66-1.13)
rs9292777	5	5p13.1	**0.0027**	0.057	0.76 (0.58-1.01)	**0.0093**	**0.66 (0.49-0.91)**
rs17234657	5	5p13.1	**0.0034**	**0.0025**	**1.64 (1.19-2.28)**	0.095	1.36 (0.95-1.95)
rs13361189	5	IRGM	**0.015**	0.12	1.41 (0.91-2.17)	**0.024**	**1.65 (1.07-2.56)**
rs4958847	5	IRGM	0.053	0.48	1.15 (0.78-1.68)	**0.018**	**1.56 (1.08-2.26)**
rs6887695	5	IL12B	**0.023**	0.17	1.21 (0.92-1.59)	**0.036**	**1.35 (1.02-1.80)**
rs2522057	5	IBD5	0.78	0.86	0.98 (0.77-1.25)	0.51	1.09 (0.84-1.41)
rs2165047	10	DLG5	**0.012**	**0.0074**	**1.42 (1.10-1.85)**	0.25	1.19 (0.89-1.58)
rs10883365	10	NKX2-3	**0.019**	**0.021**	**1.35 (1.05-1.73)**	0.31	1.15 (0.88-1.50)
rs3936503	10	CCNY	**0.032**	0.29	1.15 (0.88-1.50)	**0.022**	**1.38 (1.05-1.81)**
rs10761659	10	10q21	0.15	0.30	0.88 (0.68-1.13)	0.33	0.87 (0.67-1.14)
rs916977	15	HERC2	0.76	0.33	0.79 (0.50-1.27)	0.50	1.16 (0.75-1.80)
1007fsinsC	16	NOD2	**6.42E-10**	**1.46E-04**	**3.07 (1.67-5.64)**	**1.13E-10**	**5.19 (2.99-9.03)**
G908R	16	NOD2	**3.17E-09**	**1.77E-08**	**5.54 (2.84-10.77)**	**1.03E-05**	**4.54 (2.18-9.46)**
R702W	16	NOD2	**0.0065**	**0.0086**	**1.83 (1.16-2.88)**	0.15	1.47 (0.87-2.48)
rs2542151	18	PTPN2	0.23	**0.040**	**1.38 (1.01-1.89)**	0.80	0.95 (0.66-1.37)

Significant associations are depicted in bold.

CD - Crohn's disease; Chr - chromosome; CI - confidence interval; OR - odds ratio; SNP - single nucleotide polymorphism.

Table 3. Allelic association and minor allele frequencies (MAFs) for 19 CD associated SNPs for smoking CD patients (n=164) compared to non-smoking CD patients (n=141)

rs number	Chr	Locus	Smokers vs non-smokers (P value)	MAF smokers	MAF non-smokers	OR smokers vs non-smokers (95% CI)
rs11209026	1	*IL23R*	0.60	0.02	0.01	1.46 (0.35-6.17)
rs7517847	1	*IL23R*	0.82	0.36	0.35	1.04 (0.74-1.46)
rs12035082	1	1q24	0.92	0.40	0.40	1.02 (0.72-1.43)
rs2241880	2	*ATG16L1*	0.56	0.36	0.38	0.91 (0.65-1.27)
rs9292777	5	5p13.1	0.48	0.27	0.24	1.15 (0.78-1.69)
rs17234657	5	5p13.1	0.39	0.20	0.17	1.21 (0.79-1.86)
rs13361189	5	*IRGM*	0.56	0.10	0.11	0.85 (0.49-1.47)
rs4958847	5	*IRGM*	0.21	0.12	0.16	0.74 (0.46-1.19)
rs6887695	5	*IL12B*	0.54	0.32	0.34	0.89 (0.63-1.28)
rs2522057	5	*IBD5*	0.52	0.41	0.43	0.90 (0.65-1.24)
rs2165047	10	*DLG5*	0.31	0.31	0.27	1.20 (0.84-1.71)
rs10883365	10	*NKX2-3*	0.35	0.55	0.51	1.17 (0.84-1.64)
rs3936503	10	*CCNY*	0.31	0.36	0.40	0.84 (0.59-1.18)
rs10761659	10	10q21	0.99	0.43	0.43	1.00 (0.71-1.40)
rs916977	15	*HERC2*	0.20	0.07	0.11	0.68 (0.38-1.23)
1007fsinsC	16	NOD2	0.11	0.05	0.09	0.59 (0.31-1.13)
G908R	16	*NOD2*	0.61	0.05	0.05	1.22 (0.57-2.60)
R702W	16	*NOD2*	0.49	0.09	0.07	1.24 (0.66-2.33)
rs2542151	18	*PTPN2*	0.09	0.21	0.16	1.45 (0.94-2.23)

CD - Crohn's disease; Chr - chromosome; CI - confidence interval; MAF - minor allele frequency; OR - odds ratio; SNP - single nucleotide polymorphism.

In non-smoking, but not in smoking, CD patients associations with *IL23R* (rs7517847), 5p13.1 (rs9292777), *IRGM* (rs13361189 and rs4958847), *IL12B* (rs6887695) and *CCNY* (rs3936503) were found. *PTPN2* (rs2542151) was not associated in the entire cohort, but showed association in the smoking CD group and not in the non-smoking CD group. A direct within cases analysis showed no significantly differential distribution between smoking and non-smoking CD patients (table 3).

Allelic association analysis stratified for passive smoking in childhood

Next we divided CD patients according to exposure to cigarette smoke during childhood and analyzed whether genetic associations were different between cohorts of passive smoking and non-passive smoking CD patients. The results of the allelic association analysis stratified for passive smoking in childhood are shown in table 4 and supplementary table 2. In passively smoking CD patients associations with 12 SNPs located in eight CD susceptibility loci were found, including the *PTPN2* (rs2542151) locus again. However, in non-passively smoking CD patients only associations with *NOD2* (1007fsinsC and G908R) were found. The strong association in passive smokers with both SNPs in *IRGM* (rs13361189 and rs4958847) is remarkable since they were considerably less significantly associated in the entire CD cohort and not associated in the actively smoking CD cohort (see also table 2). A direct within cases analysis showed no significantly differential distribution between passively smoking and non-passively smoking CD patients.

Allelic association analysis stratified for passive smoking in childhood in CD patients with an early onset of disease

If passive smoking in childhood has a relationship with the development of CD, than this is most likely the case for patients with an early onset of CD. Therefore, we analyzed CD patients with an early onset of disease (40 years or younger) for genetic associations between the cohorts of passive smokers and non-passive smokers in childhood (table 5 and supplementary table 3). In passive smokers with an early onset the same associations were shown as for all passive smokers, except for the *IL23R* (rs7517847) and *IL12B* (rs6887695) loci. In non-passive smoking CD patients with an early onset associations with four SNPs located in three CD susceptibility loci were found, including the *IL23R* (rs7517847) and 1q24 (rs12035082) loci. A direct within cases analysis between passive smoking and non-passive smoking CD patients with an early onset showed an association with the IBD5 (rs2522057) locus (P=0.037; OR: 1.71 (95%CI: 1.03-2.85)). Furthermore, we analyzed whether passive smoking in childhood was related with a younger age at diagnosis in CD patients with the same genetic predisposition. For this purpose, we compared for each of the 14 CD loci the age at diagnosis between passive and non-passive smokers in childhood carrying at least one risk allele of the locus involved. This analysis showed no differences in age at diagnosis between passive and non-passive smokers in childhood.

Table 4. Allelic association for 19 CD associated SNPs for CD patients divided in passive smokers (n=160) and non-passive smokers in childhood (n=65) compared to controls (n=976)

rs number	Chr	Locus	Passive vs controls (*P* value)	OR passive vs controls (95% CI)	Non-passive vs controls (*P* value)	OR non-passive vs controls (95% CI)
rs11209026	1	IL23R	**6.12E-05**	**0.13 (0.04-0.42)**	0.13	0.47 (0.17-1.29)
rs7517847	1	IL23R	**0.021**	**0.73 (0.56-0.96)**	0.23	0.79 (0.54-1.16)
rs12035082	1	1q24	0.35	1.13 (0.87-1.47)	0.11	1.37 (0.93-2.02)
rs2241880	2	ATG16L1	0.18	0.84 (0.66-1.08)	0.15	0.76 (0.51-1.11)
rs9292777	5	5p13.1	**0.0044**	**0.65 (0.49-0.88)**	0.41	0.84 (0.55-1.28)
rs17234657	5	5p13.1	0.074	1.36 (0.97-1.92)	0.79	1.08 (0.62-1.87)
rs13361189	5	IRGM	**9.90E-04**	**1.94 (1.30-2.89)**	0.99	1.00 (0.47-2.12)
rs4958847	5	IRGM	**0.0029**	**1.68 (1.19-2.38)**	0.68	1.13 (0.63-2.03)
rs6887695	5	IL12B	**0.0092**	**1.42 (1.09-1.86)**	0.27	1.26 (0.83-1.89)
rs2522057	5	IBD5	0.63	1.06 (0.83-1.35)	0.28	0.81 (0.56-1.18)
rs2165047	10	DLG5	**0.025**	**1.35 (1.04-1.76)**	0.60	1.12 (0.74-1.69)
rs10883365	10	NKX2-3	0.17	1.19 (0.93-1.53)	0.55	1.46 (0.99-2.14)
rs3936503	10	CCNY	**0.023**	**1.36 (1.04-1.76)**	0.49	1.15 (0.77-1.72)
rs10761659	10	10q21	0.16	0.83 (0.65-1.08)	0.19	0.77 (0.52-1.14)
rs916977	15	HERC2	0.90	0.97 (0.63-1.51)	0.88	0.95 (0.48-1.86)
1007fsinsC	16	NOD2	**2.42E-05**	**3.38 (1.86-6.14)**	**9.18E-05**	**4.13 (1.91-8.90)**
G908R	16	NOD2	**1.50E-05**	**4.29 (2.10-8.78)**	**1.71E-04**	**5.06 (1.98-12.90)**
R702W	16	NOD2	**5.85E-04**	**2.13 (1.37-3.31)**	0.69	1.17 (0.53-2.60)
rs2542151	18	PTPN2	**0.025**	**1.42 (1.04-1.94)**	0.30	1.29 (0.80-2.08)

Significant associations are depicted in bold.

CD - Crohn's disease; Chr - chromosome; CI - confidence interval; OR - odds ratio; SNP - single nucleotide polymorphism.

Table 5. Allelic association for 19 CD associated SNPs for CD patients with an early onset divided in passive smokers (n=118) and non-passive smokers in childhood (n=48) compared to controls (n=976)

rs number	Chr	Locus	Passive vs controls (*P* value)	OR passive vs controls (95% CI)	Non-passive vs controls (*P* value)	OR non-passive vs controls (95% CI)
rs11209026	1	IL23R	**4.42E-04**	**0.12 (0.03-0.49)**	0.097	0.32 (0.08-1.32)
rs7517847	1	IL23R	0.087	0.77 (0.58-1.04)	**0.035**	**0.61 (0.38-0.97)**
rs12035082	1	1q24	0.40	1.13 (0.85-1.52)	**0.021**	**1.66 (1.08-2.56)**
rs2241880	2	ATG16L1	0.42	0.89 (0.67-1.18)	0.22	0.76 (0.48-1.18)
rs9292777	5	5p13.1	**0.0072**	**0.64 (0.46-0.89)**	0.54	0.86 (0.54-1.38)
rs17234657	5	5p13.1	0.17	1.31 (0.89-1.92)	0.90	1.04 (0.56-1.95)
rs13361189	5	IRGM	**6.34E-04**	**2.09 (1.36-3.23)**	0.96	0.98 (0.42-2.30)
rs4958847	5	IRGM	**0.0040**	**1.74 (1.19-2.54)**	0.92	1.04 (0.53-2.04)
rs6887695	5	IL12B	0.054	1.34 (0.99-1.81)	0.40	1.22 (0.77-1.94)
rs2522057	5	IBD5	0.16	1.22 (0.93-1.61)	0.14	0.71 (0.45-1.11)
rs2165047	10	DLG5	**0.015**	**1.44 (1.07-1.95)**	0.26	1.31 (0.82-2.07)
rs10883365	10	NKX2-3	0.79	1.04 (0.78-1.38)	0.12	1.38 (0.89-2.13)
rs3936503	10	CCNY	**0.0064**	**1.50 (1.12-2.01)**	0.36	1.23 (0.79-1.92)
rs10761659	10	10q21	0.22	0.84 (0.63-1.11)	0.082	0.67 (0.43-1.05)
rs916977	15	HERC2	0.76	0.92 (0.56-1.53)	0.45	0.72 (0.31-1.69)
1007finsC	16	NOD2	**2.02E-04**	**3.31 (1.70-6.44)**	**0.0021**	**3.74 (1.52-9.24)**
G908R	16	NOD2	**4.51E-06**	**4.96 (2.33-10.56)**	**1.19E-04**	**5.79 (2.11-15.88)**
R702W	16	NOD2	**0.019**	**1.84 (1.10-3.08)**	0.21	1.66 (0.74-3.71)
rs2542151	18	PTPN2	**0.040**	**1.43 (1.01-2.02)**	0.21	1.40 (0.83-2.38)

Significant associations are depicted in bold.

CD - Crohn's disease; Chr - chromosome; CI - confidence interval; OR - odds ratio; SNP - single nucleotide polymorphism.

Discussion

The aim of the current study was to explore whether there are differences in the genetic background of CD patients in relation to smoking behaviour. First, we found that many previously described genetic associations in large cohorts remain statistically significant in a relatively small subset of 310 CD patients of these described cohorts.[16,17] We then stratified these patients according to their smoking behaviour at diagnosis and secondly to their exposure to cigarette smoke during childhood. All these data were available from previous studies.[4,15-17] We found a striking difference of associated alleles in smoking CD patients compared to non smoking patients, implying a complex pattern of gene-environment interaction. However, no associations were found in the within cases analysis between smokers and non-smokers, which is probably caused by the small subgroups.

The question is how to interpret these results. This study has two disadvantages. First, the lack of smoking data of the control population limits the possibility of interaction analysis using a logistic regression model (multiplicative or statistical interaction). Therefore we could not evaluate the gene-environment interaction properly. This is an important issue in general, since most case-control studies that are performed in complex diseases lack data on environmental factors, particularly with respect to the control groups that are being used. Second, the small numbers of patients in the subgroups leading to a lack of power is another issue of concern, particularly with respect to passive smoking. In the case-control analyses of the patients exposed to cigarette smoke during childhood, the differences could be explained by chance findings due to the small number of patients in the non-passive smoking group. In the analysis stratified for active smoking at diagnosis lack of power does not seem to explain all the differences, since several loci (*IRGM, DLG5, NKX2-3*) are associated in one of the two groups, while the *P* value and OR in the other group do not show any association or even a trend towards association.

An even more intriguing finding is that *PTPN2* is not associated in the entire group ($P=0.23$) or in the non-smokers group ($P=0.79$), but is associated with CD in the smoking at diagnosis ($P=0.041$) and the passive smoking in childhood groups ($P=0.025$). In addition, in the analysis between active smokers and non-smokers the *P*-value for *PTPN2* is 0.09 (table 3) and the association will become most likely significant in larger cohorts. Association with this locus was reported for the first time by the Wellcome Trust Case Control Consortium.[21] *PTPN2*, protein tyrosine phosphatase nonreceptor type 2, is able to reverse tyrosine phosphorylation and is important for the regulation of haematopoiesis and in cytokine signalling.[22] *PTPN2* is also associated with rheumatoid arthritis (RA) and type 1 diabetes.[21] In both these autoimmune diseases, smoking is also an important environmental factor. Smoking is the best established environmental risk factor for RA[23-25] and maternal smoking in pregnancy has protective effects on type 1 diabetes.[26-28] Another tyrosine phosphatase nonreceptor, *PTPN22*, which is a critical gatekeeper of T cell receptor signalling, is associated with RA.[29] Interestingly, for this particular gene, gene-environment interaction with cigarette smoking for more than 10 pack-years is observed in RA.[30] These findings further support the idea that specific environmental factors are necessary for specific genetic variants to contribute to disease development.

Despite the limitations of the current study, it is clear that when our cohort is stratified for smoking, many different genetic associations become apparent and genes like *PTPN2* are only associated in a subgroup. This is an important finding since most genetic case-control

studies are not stratified for environmental factors and are not matched with data on environmental factors in the control cohorts. In studies with only limited sample size true genetic associations might therefore be missed. In IBD, a large part of the disease pathogenesis consists of (yet unknown) environmental factors and their interaction with the genetic background of the patient. Extrapolating our findings, even in large cohorts true moderately sized associations can be missed, when environmental factors are not taken into account.

Passive smoking in childhood has also been related to the development of CD.[5,6] Therefore we studied differences in genetic associations in CD patients divided in passive and non-passive smokers in childhood. In passively smoking CD patients associations with eight loci (*IL23R*, 5p13.1, *IRGM, IL12B, DLG5, CCNY, NOD2* and *PTPN2*) were found, while in non-passively smoking CD patients only associations with *NOD2* (1007fsinsC, G908R) were found. As mentioned before, part of this might be explained by the small subgroup of non-passively smoking CD patients (n=65) implying limited statistical power. However, several SNPs like *IRGM* (rs13361189; *P*=0.99 and rs4958847; *P*=0.68), *DLG5* (rs2165047; *P*=0.60) and *NOD2* (R702W; *P*=0.69) show no association at all and will most likely also not be associated in larger non-passively smoking CD cohorts either.

An effect of passive smoking in childhood will probably be the largest in those patients with an early onset of CD. Therefore we studied genetic associations between passive and non-passive smokers with onset of CD before the age of 40 years. In passive smokers with an early onset the same associations were shown as for all passive smokers, except for the *IL23R* (rs7517847) and *IL12B* (rs6887695) loci. Interesting are the findings in the non-passively smoking CD patients with an early onset when compared with non-passive smokers in the entire cohort. In non-passive smokers with an early onset associations with *IL23R* (rs7517847), 1q24 (rs12035082) and *NOD2* (1007finsC and G908R) were found, while in non-passive smokers in the entire cohort only associations with *NOD2* were found. This might suggest that in non-passive smokers with an early onset genetic background is more important than for non-passive smokers with a late onset of CD. However, these results are of course for a great part scattered by the decrease in sample size and further research is needed in larger cohorts about the true interaction between genetic variants, passive smoking in childhood and early age at diagnosis of CD.

In conclusion, we found different genetic associations in different groups according to active smoking at diagnosis and passive smoking in childhood. Part of the difference can be explained by the lack of power in this relatively small CD cohort. However, it is clear that there is an interaction between genes and the environment. Furthermore, our data suggest that in order to find all CD associated genes, particularly those with low odds ratios, we need to stratify patient and control cohorts for smoking behaviour, including passive smoking in childhood. Therefore, genetic studies in CD, and probably also in ulcerative colitis and RA, should be stratified for smoking behaviour, especially in cohorts of limited sample size. Otherwise moderately associated genes like *PTPN2* can be missed.

Supplementary table 1. Allelic association and minor allele frequencies (MAFs) for 19 CD associated SNPs for controls (n=976) compared to all CD patients (n=310)

rs number	Chr	Locus	CCA (P value)	MAF controls	MAF cases	OR (95% CI)
rs11209026	1	IL23R	**4.45E-07**	0.07	0.01	**0.19 (0.09-0.39)**
rs7517847	1	IL23R	**0.0060**	0.43	0.36	**0.75 (0.61-0.92)**
rs12035082	1	1q24	**0.044**	0.35	0.40	**1.23 (1.01-1.51)**
rs2241880	2	ATG16L1	**0.046**	0.42	0.37	**0.82 (0.68-1.00)**
rs9292777	5	5p13.1	**0.0027**	0.33	0.26	**0.71 (0.57-0.89)**
rs17234657	5	5p13.1	**0.0034**	0.13	0.18	**1.48 (1.14-1.93)**
rs13361189	5	IRGM	**0.015**	0.07	0.10	**1.52 (1.08-2.14)**
rs4958847	5	IRGM	0.053	0.11	0.14	1.33 (1.00-1.78)
rs6887695	5	IL12B	**0.023**	0.28	0.33	**1.28 (1.04-1.58)**
rs2522057	5	IBD5	0.78	0.41	0.42	1.03 (0.85-1.24)
rs2165047	10	DLG5	**0.012**	0.24	0.29	**1.30 (1.06-1.60)**
rs10883365	10	NKX2-3	**0.019**	0.48	0.54	**1.27 (1.04-1.54)**
rs3936503	10	CCNY	**0.032**	0.33	0.38	**1.25 (1.02-1.54)**
rs10761659	10	10q21	0.15	0.46	0.42	0.87 (0.71-1.06)
rs916977	15	HERC2	0.76	0.09	0.09	0.95 (0.67-1.34)
1007finsC	16	NOD2	**6.42E-10**	0.02	0.07	4.07 (2.52-6.56)
G908R	16	NOD2	**3.17E-09**	0.01	0.05	**4.98 (2.77-8.96)**
R702W	16	NOD2	**0.0065**	0.05	0.08	**1.67 (1.15-2.42)**
rs2542151	18	PTPN2	0.23	0.16	0.19	1.17 (0.91-1.51)

Significant associations are depicted in bold.
CD - Crohn's disease; Chr - chromosome; CCA - case control allelic association analysis; CI - confidence interval; MAF - minor allele frequency; OR - odds ratio; SNP - single nucleotide polymorphism.

Supplementary table 2. Allelic associations and minor allele frequencies (MAFs) for 19 CD associated SNPs for passively smoking CD patients (n=160) compared to non-passively smoking CD patients (n=65) in childhood

rs number	Chr	Locus	MAF passive smokers	MAF non-passive smokers	Passive smokers vs non-passive smokers (P value)	OR passive vs non-passive smokers (95% CI)
rs11209026	1	IL23R	0.01	0.03	0.08	0.29 (0.06-1.30)
rs7517847	1	IL23R	0.35	0.37	0.74	0.93 (0.60-1.44)
rs12035082	1	1q24	0.38	0.43	0.39	0.83 (0.53-1.28)
rs2241880	2	ATG16L1	0.38	0.35	0.63	1.11 (0.72-1.72)
rs9292777	5	5p13.1	0.24	0.29	0.32	0.78 (0.48-1.27)
rs17234657	5	5p13.1	0.17	0.14	0.45	1.27 (0.69-2.33)
rs13361189	5	IRGM	0.13	0.07	0.10	1.93 (0.87-4.28)
rs4958847	5	IRGM	0.17	0.12	0.22	1.49 (0.79-2.81)
rs6887695	5	IL12B	0.35	0.32	0.59	1.13 (0.72-1.80)
rs2522057	5	IBD5	0.43	0.36	0.22	1.31 (0.85-2.01)
rs2165047	10	DLG5	0.30	0.26	0.42	1.21 (0.76-1.93)
rs10883365	10	NKX2-3	0.52	0.57	0.37	0.82 (0.53-1.27)
rs3936503	10	CCNY	0.40	0.36	0.48	1.18 (0.75-1.85)
rs10761659	10	10q21	0.41	0.39	0.72	1.08 (0.70-1.69)
rs916977	15	HERC2	0.09	0.09	0.95	1.02 (0.48-2.20)
1007finsC	16	NOD2	0.06	0.07	0.63	0.82 (0.36-1.87)
G908R	16	NOD2	0.04	0.05	0.75	0.85 (0.31-2.29)
R702W	16	NOD2	0.10	0.06	0.17	1.81 (0.77-4.27)
rs2542151	18	PTPN2	0.22	0.20	0.72	1.10 (0.65-1.88)

CD - Crohn's disease; Chr - chromosome; CI - confidence interval; MAF - minor allele frequency; OR - odds ratio; SNP - single nucleotide polymorphism.

Supplementary table 3. Allelic associations and minor allele frequencies (MAFs) for 19 CD associated SNPs for passively smoking CD patients (n=118) compared to non-passively smoking CD patients (n=48) in childhood with an early onset of disease

rs number	Chr	Locus	MAF passive smokers	MAF non-passive smokers	Passive smokers vs non-passive smokers (P value)	OR passive vs non-passive smokers (95% CI)
rs11209026	1	IL23R	0.01	0.02	0.32	0.38 (0.05-2.72)
rs7517847	1	IL23R	0.36	0.31	0.37	1.27 (0.75-2.14)
rs12035082	1	1q24	0.38	0.48	0.13	0.68 (0.42-1.12)
rs2241880	2	ATG16L1	0.39	0.35	0.53	1.18 (0.71-1.96)
rs9292777	5	5p13.1	0.24	0.30	0.28	0.74 (0.42-1.28)
rs17234657	5	5p13.1	0.17	0.14	0.53	1.25 (0.62-2.53)
rs13361189	5	IRGM	0.14	0.07	0.10	2.14 (0.86-5.33)
rs4958847	5	IRGM	0.18	0.11	0.17	1.68 (0.80-3.52)
rs6887695	5	IL12B	0.34	0.32	0.72	1.10 (0.65-1.86)
rs2522057	5	IBD5	0.46	0.33	**0.04**	**1.71 (1.03-2.85)**
rs2165047	10	DLG5	0.31	0.29	0.71	1.11 (0.65-1.87)
rs10883365	10	NKX2-3	0.49	0.56	0.26	0.75 (0.46-1.24)
rs3936503	10	CCNY	0.42	0.38	0.45	1.22 (0.73-2.02)
rs10761659	10	10q21	0.42	0.36	0.40	1.24 (0.75-2.07)
rs916977	15	HERC2	0.09	0.07	0.61	1.28 (0.49-3.32)
1007fsinsC	16	NOD2	0.06	0.07	0.81	0.88 (0.33-2.40)
G908R	16	NOD2	0.05	0.06	0.78	0.86 (0.29-2.54)
R702W	16	NOD2	0.09	0.08	0.83	1.11 (0.45-2.74)
rs2542151	18	PTPN2	0.22	0.22	0.95	1.02 (0.56-1.85)

Significant associations are depicted in bold.
CD - Crohn's disease; Chr - chromosome; CI - confidence interval; MAF - minor allele frequency; OR - odds ratio; SNP - single nucleotide polymorphism.

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Chapter 6

Protective effects of cigarette smoke on ulcerative colitis are not related to induced heme oxygenase 1 expression in colonic epithelial cells

F. van der Heide, E.M.J. van der Logt, T. Blokzijl, J.H. Kleibeuker, K.N. Faber, G. Dijkstra

Submitted.

Abstract

Introduction:
Smoking is beneficial for ulcerative colitis (UC), but underlying mechanisms are unknown. Induction of heme oxygenase 1 (HO-1) was beneficial in animal models of intestinal injury. Cigarette smoke (CS) can induce HO-1, but the effect of smoking on intestinal HO-1 is unknown. We studied the effects of smoking on colonic HO-1 expression *in vitro*, in animals and in humans.

Methods:
DLD-1 cells were incubated with cigarette smoke extract (CSE). Mice were exposed to normal air or to CS for eight days. Colon biopsies of healthy human non-smokers and smokers were collected. HO-1 expression was evaluated by quantitative polymerase chain reaction (qPCR), western blotting and immunohistochemistry. Cytochrome P450 1A1 (CYP1A1) expression (qPCR) was used as a positive control for exposure of the colon to CS.

Results:
CSE dose-dependently induced HO-1 and CYP1A1 mRNA expression in DLD-1 cells. In mice and humans, colonic CYP1A1 mRNA expression was increased in subjects exposed to CS, but colonic HO-1 mRNA and protein expression were equal between controls and subjects exposed to CS.

Conclusion:
CS does induce a CYP1A1 response, but not a HO-1 stress response in colonic epithelial cells. This indicates that CS does not exert its beneficial effect on UC via up-regulation of colonic HO-1, but could trigger other protective mechanisms locally in the colon.

Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by chronic relapsing inflammation of the colon. Smoking is an important environmental factor in UC. Patients with UC are less likely to be smokers than controls,[1-5] implying that smoking protects against the development of UC. Besides this protective effect on the development, smoking also has beneficial effects on the disease course of UC. Smoking was associated with less hospitalizations,[5-8] and decreased need for steroids [5,8,9] and surgery.[5-10] Although the beneficial effect of smoking in UC is already known for years, it is still unknown which of the more than 4,000 components in cigarette smoke (CS) is responsible for this effect and which protective pathways are involved.

One of the pathways involved could be heme oxygenase (HO). HO is the rate-limiting enzyme involved in the breakdown of heme, yielding the end-products biliverdin, Fe^{2+} and carbon monoxide (CO). Heme causes oxidative stress, while all three end-products have anti-oxidative, anti-apoptotic and anti-inflammatory properties.[11-17] Three isoforms of HO are identified: HO-1, HO-2 and HO-3, of which HO-1 is the only inducible form. It can be induced by heme, but also by oxidative stress, ischemia-reperfusion, prostaglandins, hormones, hypoxia and inflammatory cytokines.[18-21] Induction of HO-1 was beneficial for the intestines in several models of oxidative injury and inflammation; it protected the gut against ischemia-reperfusion injury in rats,[22,23] ameliorated colitis in IL-10 knock-out mice,[24] and protected against intestinal damage in TNBS-induced colitis in rats [25] and in DSS-induced colitis in mice.[26,27] In contrast, inhibition of HO-1 expression enhanced intestinal inflammation and injury in TNBS-induced colitis in rats [28] and in DSS-induced colitis in mice.[29] Taken together, these observations implicate that increased expression of HO-1 is a protective defence mechanism against oxidative and inflammatory injury of the intestines. Therefore, induction of HO-1 could be beneficial for UC patients by protecting against development of UC and/or by ameliorating inflammation in established UC.

Considering the beneficial effects of smoking on UC and of HO-1 induction on intestinal inflammation, it is interesting that smoking is also able to induce HO. Exposure to CS increased HO-1 expression in lung epithelial cells and/or alveolar macrophages in both *in vitro* [30,31] and animal [31,32] studies. In humans, smokers had increased expression of HO-1 in lung tissue [33] and gingival tissue.[34] In addition, CS also induced HO-1 in other cultured human cells like monocytes [35,36] and vascular endothelial cells.[36] However, the effect of CS on intestinal HO-1 expression is unknown *in vitro*, in animals and in humans.

The aim of this study is to explore whether CS increases the colonic expression of HO-1. For this purpose, we studied the effects of smoking *in vitro*, in mice and in humans.

Methods

Materials

All supplies for cell culture experiments and for quantitative polymerase chain reaction (qPCR) analysis were obtained from Invitrogen (Breda, The Netherlands), unless stated otherwise. For 100% cigarette smoke extract (CSE), 25 ml serum-free culture medium was saturated with smoke from two Kentucky 3R4F research cigarettes (University of Kentucky, Lexington, KY, USA) using a peristaltic pump (Watson-Marlow, Rotterdam, The Netherlands).

Cell culture experiments

To examine whether cigarette smoke affects colonic HO-1 expression *in vitro*, cells of the human colon adenocarcinoma cell line DLD-1 were exposed to CSE. Cells were cultured in RPMI-1640 glutamax medium supplemented with 10% v/v heat-inactivated fetal calf serum, penicillin (50 U/mL), streptomycin (50 μg/mL) and fungizone (5 μg/mL) in a humidified incubator at 37°C in an atmosphere containing 5% carbon dioxide. Harvested cells were placed on fresh serum-free culture medium and then incubated with 0, 5, 10, 20, 30, 50 or 100% CSE for 9 h. Incubating DLD-1 cells with 50 and 100% CSE caused significant cell death and therefore we only report the data of incubation with 0-30% CSE. All experiments were performed in triplicate. Experiments were started within one hour after preparation of CSE. At the end of the experiments, each well was washed with HBSS and harvested in Trizol or lysisbuffer for qPCR and western blot analyses, respectively. Samples were stored at -80°C until further use.

Animal experiments

To examine whether cigarette smoke affects colonic HO-1 expression *in vivo,* female BALB/c mice (19-21 g; Harlan, Boxmeer, The Netherlands) were exposed to CS. Mice were housed under standard laboratory conditions with free access to standard laboratory chow and water. Mice were divided into two groups: 1) exposure to normal air (n=7) and 2) exposure to a mixture of 8% CS and normal air (n=7). The 8% CS/air mixture was obtained by smoking Kentucky 3R4F research cigarettes using a peristaltic pump (Watson-Marlow). Mice were exposed twice daily for 30 minutes (four cigarettes per session with three minutes normal air after each smoked cigarette) for 8 days in total. In parallel, control mice were exposed to normal air following the same schedule. During exposure, both groups of mice were placed in an air-sealed perspex box connected to an air-exhaust ventilator. The experimental design was approved by the ethical committee of animal welfare of our hospital.

The mice were weighed daily, and at day 8 anaesthetised with isoflurane/O_2 and sacrificed by heart puncture. Blood samples were collected to assess effective smoke exposure by measuring cotinine levels. Plasma samples were analysed with a certified liquid chromatography and tandem mass spectrometry procedure (TSQ Quantum, Thermo Fisher Scientific, Wilmington, DE, USA) at the department of Hospital and Clinical Pharmacy of our hospital. For comparison, in humans a plasma cotinine level $\geq$15 μg/l is indicative of active smoking.[37] The colon was removed and the lumen was washed with PBS. Then the colon was divided into samples for qPCR, western blotting and immunohistochemistry, and immediately snap-frozen in liquid nitrogen or fixed with formaldehyde, respectively. Next, samples were stored at -80°C until further processing or embedded in paraffin for immunohistochemistry.

Human study

To examine whether cigarette smoke affects colonic HO-1 expression in humans, we examined HO-1 levels in colon biopsies of healthy smokers (n=9) and non-smokers (n=9) obtained during colonoscopy. Non-smokers were defined as those who quitted smoking at least one year ago or never smoked. We excluded subjects: 1) on steroids, immunosuppression or omeprazole (the last is capable of inducing cytochrome P450 1A1 (CYP1A1))[38,39], 2) after bowel resection, or 3) with abnormal findings other than diverticulosis, hyperplastic polyps or adenomas during colonoscopy, or with histological signs of inflammation. Characteristics of the participants are described in Table 1. All participants gave informed consent and the methods were approved by the medical ethics committee of our hospital.

Table 1. Characteristics of the healthy smokers and non-smokers

	Smokers (n=9)	Non-smokers (n=9)
Female/male	4/5	4/5
Median age (range)	44 (24-56)	49 (27-77)
Smoking behaviour:		
smoker	10 (100%)	0
former smoker	0	4 (44%)
never smoker	0	5 (56%)
Median number of years smoked (range)	30 (7-40)	
Median number of cigarettes/day (range)	17 (4-25)	
Indication for colonoscopy:		
adenoma surveillance	2 (22%)	2 (22%)
exclude inflammatory bowel disease	4 (44%)	6 (67%)
other	3 (33%)	1 (11%)

Thirty minutes before colonoscopy smokers smoked one Kentucky 3R4F research cigarette. Just before colonoscopy, a venous blood sample was taken to measure carboxyhemoglobulin to confirm smoking status. Carboxyhemoglobulin was measured by the ABL800 FLEX blood gas analyzer (Radiometer, Zoetermeer, The Netherlands). Reference values for non-smokers range from 0.5% to 1.5% (http://www.fk.cvz.nl). During colonoscopy biopsies were taken in the ascending colon, except in two subjects they were taken in the transverse colon. Two specimens for qPCR and western blotting were immediately snap-frozen in liquid nitrogen and stored at -80°C until further processing, and two specimens for immunohistochemistry were fixed with formaldehyde and embedded in paraffin.

RNA isolation and quantitative polymerase chain reaction (qPCR)

For isolating RNA from DLD-1 cells and from colon samples of mice and humans, we used Trizol according to the manufacturer's instructions. RNA content was quantified using NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). Reverse transcription and qPCR conditions were performed as described before.[40] For qPCR we designed sense and antisense primers and fluorogenic probes (Eurogentec, Herstal, Belgium) for HO-1, inducible nitric oxide synthase (iNOS), CYP1A1 and 18S using Primer Express 2.0 software (Applied Biosystems, Foster City, CA, USA). For primers and probes see supplementary Table 1. We performed qPCR for iNOS to exclude inflammation not detected by the pathologist, since iNOS expression is increased in inflammation.[16] CYP1A1 is an enzyme involved in the metabolism of a number of procarcinogens, including polycyclic aromatic hydrocarbons found in CS. Smokers had increased CYP1A1 activity in the duodenum,[39] and therefore colonic CYP1A1 expression (qPCR) was studied as a positive control for exposure of the colon to CS. Fluorescence was measured using 7900 HT Fast RT-PCR System (Applied Biosystems). Each sample was analyzed in duplicate (ABI PRISM SDS 2.1 software, Applied Biosystems). Finally, the gene of interest was normalized to 18S ($2^{-\Delta Ct}$ method).

Western blot analysis

DLD-1 cells were lysed by four cycles of freeze-thawing (liquid nitrogen-37 °C). After ten minutes centrifugation at 13.000 g supernatant was collected. Colon specimens from mice and humans were homogenized in lysis buffer by 25 strokes with a plastic pestle and supernatant was obtained after ten minutes centrifugation at 13.000 *g*. Protein concentrations were measured using the BioRad D_C protein assay (Bio-Rad, Veenendaal, The Netherlands).

For analyzing protein expression we performed western blots. Twenty microgram of protein was fractioned on a 10% SDS-PAGE gel and transferred to a nitrocellulose membrane (Hybond-ECL, Amersham Biosciences, GE Healthcare, Diegem, Belgium) by semi-dry blotting. Membranes were stained with Ponceau-S for transfer control. Next, the membranes were blocked with 2% milk/0.5% BSA (ELK, Campina, Zaltbommel, The Netherlands) in PBS/0.1% Tween-20 (PBS-T, Sigma-Aldrich). For HO-1 detection we used a monoclonal antibody #OSA-110 (1:1000, StressGen, Victoria, British Columbia, Canada) as primary antibody. After washing with PBS-T, blots were incubated with the secondary antibody horseradish peroxidase-labeled goat anti-rabbit IgG (1:2000, Dako, Glostrup, Denmark). Blots were washed with PBS-T and finally with PBS, and developed by exposure to ECL substrate (SuperSignal West Dura, Pierce/Perbio Sciences, Etten-Leur, The Netherlands). Blots were stripped in PBS-T in 0.1% SDS at 65°C for 30 min and re-probed with a GAPDH antibody and used as loading control.

Immunohistochemistry analysis

Four μm thick slices were cut from colon tissue of mice and humans. For HO-1 detection antigen-retrieval was performed in EDTA-buffer at 300 Watt for 15 min in a microwave oven. Slides were incubated with an anti-HO-1 monoclonal antibody (1:200, #OSA-110, StressGen) as described before.[16] Endogenous peroxidase was blocked with 0.3% hydrogen peroxide. Peroxidase-conjugated rabbit anti-mouse Ig and peroxidise-conjugated mouse anti-rabbit were used as secondary and tertiary antibodies (both 1:50 in 1% BSA/PBS containing 1% human serum, Dako). Colour was developed using 3-3-diaminobenzidine tetrahydrochloridehydrate. Slides were counter-stained with hematoxylin. Negative control stainings were performed by omitting the anti-HO-1 antibody. Images were taken with a Leica DM LB microscope (Leica, Wetzlar, Germany).

Statistical analysis

Descriptive variables are presented as medians (range) or as means (standard deviation), and categorical variables as frequencies with percentages. Differences between groups were compared with the Mann-Whitney U-test. A p-value <0.05 was considered statistically significant. The data were analyzed using GraphPad Prism 5.00 software (GraphPad software, San Diego, CA, USA).

Results

Effect of cigarette smoke (CS) on HO-1 expression in DLD-1 cells

First, we studied the effects of CSE on colonic HO-1 expression *in vitro*. CSE dose-dependently induced HO-1 mRNA levels in DLD-1 cells, from 1.6-fold at 5% CSE to 80-fold at

30% CSE (Figure 1A). CSE also dose-dependently induced HO-1 protein expression as determined by Western blot analysis (Figure 1B). Summarizing, CS increased colonic HO-1 expression *in vitro*.

Effect of cigarette smoke on colonic HO-1 expression in mice

Second, we studied the effects of CS on colonic HO-1 expression in mice. Analysis of the blood samples revealed a median cotinine level of 65 μg/l (range 45-112 μg/l) in mice exposed to CS and <5μg/l in control mice. In contrast to our hypothesis, colonic HO-1 mRNA levels were equal between mice exposed to normal air and to CS (Figure 2). No significant amounts of HO-1 were detected by Western blotting and immunohistochemistry, confirming the findings by qPCR (data not shown). Clinically, we observed no difference in body weight between mice exposed to normal air and to CS. The iNOS mRNA levels were equal between both groups of mice, implying no differences in colonic inflammation (data not shown). Summarizing, cotinine levels were high in mice exposed to CS, but CS had no effect on colonic HO-1 expression in mice.

Effect of cigarette smoke on colonic HO-1 expression in humans

Third, we studied the effects of CS on colonic HO-1 expression in humans. Nine smokers with median smoking time of 30 years and smoking intensity of median 17 cigarettes per day were compared to nine non-smokers (Table 1). In non-smokers median carboxyhemoglobulin levels were 0.8% (0.5%-1.1%) and in smokers 5.1% (range 3.0%-6.2%) just before

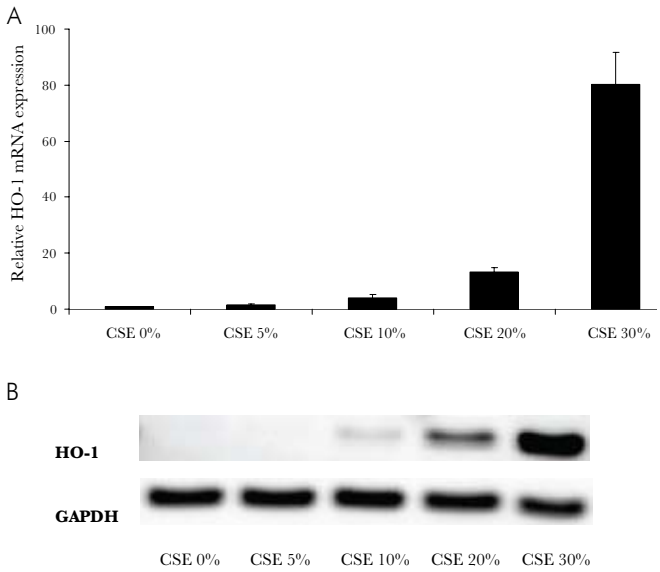


Figure 1. (A) Heme oxygenase 1 (HO-1) mRNA expression levels in DLD-1 cells incubated with cigarette smoke extract (CSE) 0, 5, 10, 20 or 30% for 9 h. The HO-1 mRNA expression was normalized to the expression of 18S. CSE dose-dependently induced HO-1 mRNA levels. Data are presented as mean of three independent experiments ± SD. (B) A representative Western blot of HO-1 protein expression in DLD-1 cells incubated with CSE 0, 5, 10, 20 or 30% for 9 h. GAPDH is used as internal control. CSE dose-dependently induced HO-1 protein levels.

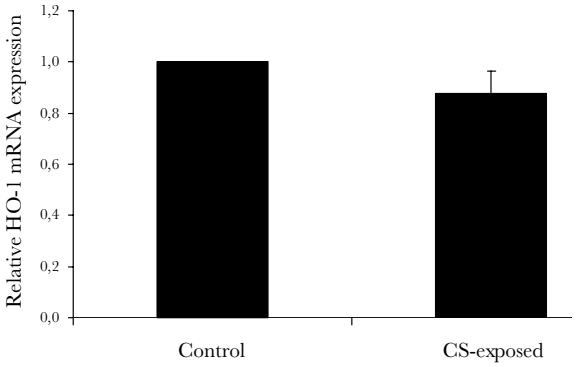
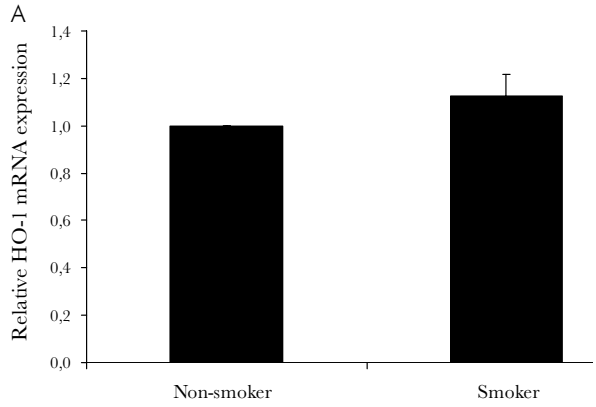


Figure 2. Colonic heme oxygenase 1 (HO-1) mRNA expression levels in mice exposed to normal air (controls) and to cigarette smoke for eight days. The HO-1 mRNA expression was normalized to the expression of 18S. HO-1 mRNA levels were equal between mice exposed to normal air and to cigarette smoke. Data are expressed as mean (n=7) ± SD.

colonoscopy. In line with our findings observed in mice, levels of colonic HO-1 mRNA were equal between healthy non-smokers and smokers (Figure 3A). HO-1 protein expression on Western blot was very low in both smokers and non-smokers (data not shown). Immunohistochemistry was in line with the findings on mRNA level, with no differences in HO-1 staining between non-smokers and smokers (Figure 3B). HO-1 staining was mainly localized in epithelial cells, and in a few inflammatory cells. Summarizing, we found no effects of CS on colonic HO-1 expression in humans.

Effect of cigarette smoke on colonic CYP1A1 expression

Finally, we studied the effects of CS on colonic CYP1A1 expression as a positive control for exposure of the colon to CS. We examined the colonic CYP1A1 mRNA expression *in vitro*, in mice and in humans exposed to CS. In DLD-1 cells exposed to CSE a dose-dependent induction of CYP1A1 mRNA levels was observed (Figure 4A). The highest up-regulation was seen with 10% (1198-fold) and 20% (991-fold) CSE. In mice exposed to CS (Figure 4B) and in smoking humans (Figure 4C) CYP1A1 mRNA expression was also increased (7-fold and 26-fold, respectively) compared to subjects not exposed to CS. Summarizing, CS increased colonic CYP1A1 expression *in vitro,* in mice and in humans.



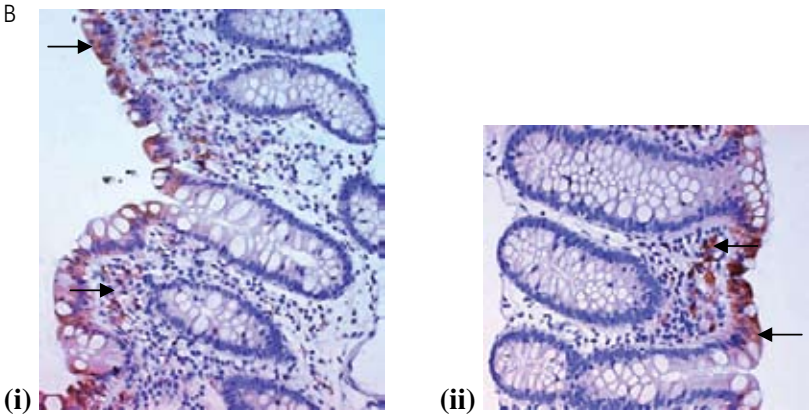


Figure 3. (A) Heme oxygenase 1 (HO-1) mRNA expression levels in colon biopsies of human non-smokers and smokers. The HO-1 mRNA expression was normalized to the expression of 18S. HO-1 mRNA levels were equal between non-smokers and smokers. Data are expressed as mean (n=9 and 10) ± SD. (B) Immunohistochemistry for HO-1 in colon biopsies of human non-smokers (i: 200x) and smokers (ii: 200x). Staining of HO-1 **(arrows)** is mainly localized in the epithelial cells on the surface, and in a few inflammatory cells scattered throughout the lamina propria. There are no differences in staining between non-smokers and smokers.

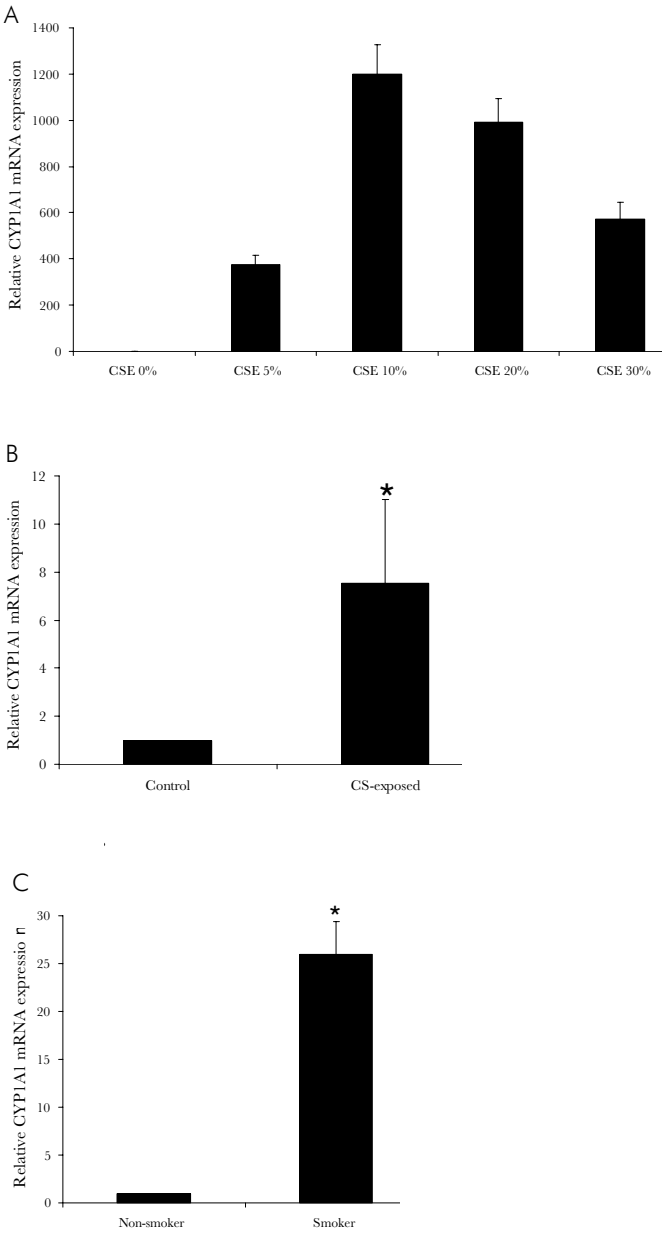


Figure 4. Cytochrome P450 1A1 (CYP1A1) mRNA expression levels in (A) DLD-1 cells incubated with cigarette smoke extract (CSE 0, 5, 10, 20 or 30%) for 9 h, (B) colons of mice exposed to normal air (controls) and to cigarette smoke for eight days, and (C) colon biopsies of human non-smokers and smokers. The CYP1A1 mRNA expression was normalized to the expression of 18S. CYP1A1 mRNA levels were increased in DLD-1 cells, mice and humans exposed to cigarette smoke. Data are expressed as mean ± SD. * P <0.001 compared with controls (= non-smokers).

Supplementary table 1. Primers and probes used for quantitative real time PCR

	Forward primers 5'→3'	Reversed primer 5'→3'	Probe 5'→3'
Human:			
18S	CGG CTA CCA CAT CCA AGG A	CCA ATT ACA GGG CCT CGA AA	CGC GCA AAT TAC CCA CTC CCG A
HO-1	GAC TGC GTT CCT GCT CAA CAT	GCT CTG GTC CTT GGT GTC ATG	TCA GCA GCT CCT GCA ACT CCT CAA AGA G
iNOS	GGC TCA AAT CTC GGC AGA ATC	GGC CAT CCT CAC AGG AGA GTT	TCC GAC ATC CAG CCG TGC CAC
CYP1A1	AGA AGA TGG TCA AGG AGC ACT ACA A	GCT CAA TCA GGC TGT CTG TGA T	CTT TGA GAA GGG CCA CAT CCG GG
Mice:			
18S	CGG CTA CCA CAT CCA AGG A	CCA ATT ACA GGG CCT CGA AA	CGC GCA AAT TAC CCA CTC CCG A
HO-1	CAC AGG GTG ACA GAA GAG GCT AA	CTG GTC TTT GTG TTC CTC TGT CAG	CAG CTC CTC AAA CAG CTC AAT GTT GAG C
iNOS	CTA TCT CCA TTC TAC TAC TAC CAG ATC GA	CCT GGG CCT CAG CTT CTC AT	CCC TGG AAG ACC CAC ATC TGG CAG
CYP1A1	CCT TCC GGC ATT CAT CCT T	TAT AGA AGC CAT TCA GAC TTG TAT CTC TTG	CCT TCA CCA TCC CCC ACA GCA CC

CYP1A1, cytochrome P450 1A1; HO-1, heme oxygenase 1; iNOS, inducible nitric oxide synthase.

Discussion

Smoking has beneficial effects on UC, but the pathways influenced by CS are unknown. Two findings suggest the putative involvement of the HO-1 pathway. First, induction of HO-1 was beneficial in animal models of intestinal injury.[22-27] Second, cigarette smoke is able to induce HO-1 in several different human cells and tissues.[30,31,33-36] However, the effect of CS on intestinal epithelial cells was so far unknown. Therefore, we studied the effects of CS on colonic HO-1 *in vitro*, in mice and in humans. In the animal experiments plasma cotinine levels were highly indicative of active smoking and in the human experiments smoking exposure was proven by measuring carboxyhemoglobulin levels. In addition, the number of cigarettes smoked by our study objects (median 17 per day) is in the therapeutic range of UC, since the studies with a positive effect of smoking on UC had a median number of cigarettes comparable or lower than 17 cigarettes per day.[5-7] We hypothesized that CS increases the colonic expression of HO-1, implying that the beneficial effect of smoking in UC could act through the HO-1 pathway. However, our findings did not support our hypothesis. CSE induced HO-1 *in vitro*, but there were no differences in HO-1 expression between smokers and non-smokers in mice and humans.

The beneficial effects of HO-1 on colitis have been studied by different approaches. Paul *et al*. studied the effects of HO-1 induction on an experimental colitis model in mice and showed that induction before the onset of inflammation led to reduced colonic inflammation.[26] These results suggested a protective effect of HO-1 induction on the development of colitis, which is in line with the beneficial effect of smoking on the development of UC.[1-5] However, HO-1 induction after the onset of acute colitis or during chronic colitis was not effective and they concluded that induction of HO-1 may not be a promising approach in chronic UC.[26] This is not in line with the beneficial effects of smoking on UC after diagnosis.[5-10]

Previously, we studied the regulation and interaction of HO-1 and iNOS in response to oxidative stress and inflammation in vitro and in rats.[16] Oxidative stress induced HO-1 in intestinal epithelial and inflammatory cells, but prevented iNOS induction in an NF-κB-dependent manner. Inflammation caused by a cytokine mixture of IL-1β, IFN-γ and TNF-α induced iNOS, but not HO-1. Furthermore, the HO-1 end-product CO inhibited the cytokine mixture induced iNOS expression. These data demonstrated opposite regulation of HO-1 and iNOS in intestinal epithelial cells in response to oxidative stress and cytokine exposure, suggesting that HO-1 (activator protein-1 driven) and iNOS (NF-κB driven) represent mutually exclusive survival mechanisms in intestinal epithelial cells.

The intestinal HO-1 expression in patients with UC has also been studied. HO-1 expression was increased in active UC compared to inactive disease and healthy controls.[41] Another study showed increased HO-1 expression in patients with UC and other conditions of chronic intestinal inflammation as Crohn's disease, intestinal ischemia and diverticulitis.[26] Finally, intestinal HO-1 expression was increased in biopsies from inflamed colon in UC patients compared to colon biopsies from normal mucosa of subjects with colon cancer.[42] No clear consensus is present about the cell type-specific expression of HO-1 in the intestinal mucosa. Two studies showed HO-1 staining in both inflammatory and epithelial cells,[26,41] but in a recent study no significant amounts of HO-1 were detected in epithelial cells.[42] In contrast, we found HO-1 staining in normal mucosa predominantly in epithelial cells, and in a few inflammatory cells.

Our data exclude a local effect of smoking on intestinal HO-1, since we found no dif-

ferences in intestinal HO-1 expression between smokers and non-smokers. Nevertheless, smoking is able to trigger other protective processes localized in the colon, since we observed that smoking inhaled via the lungs finally reaches the colon and increases the expression of colonic CYP1A1 in smokers compared to non-smokers. Still, our findings do not fully exclude an interaction between smoking and the HO-1 pathway as being beneficial for UC patients. Smoking could act its beneficial effects on UC through a systemic up-regulation of HO-1. Healthy smokers have increased HO-1 expression in the lungs[33] and in the gingival tissue[34] leading to an increase in the anti-oxidative, anti-apoptotic and anti-inflammatory end-products biliverdin, Fe^{2+} and CO.[11-17] Especially the beneficial role of CO seems promising. Exposure to CO ameliorated chronic colitis in mice[24] and protected the transplanted intestine against cold ischemia/reperfusion injury in rats.[43] Since smoking causes chronic exposure to CO,[14,17] smoking could also exert its beneficial role in UC directly via CO and thus independent of HO-1.

It is important to note the putative limitations of our study. The mouse model for studying the effects of smoking may not be a good model for active smoking. Whole body exposure is actually more a model for passive smoking. However, this model is less stressful for mice than the nose-only exposure model (active smoking), where mice are put in strains and are not able to move. Furthermore, the increased colonic CYP1A1 expression in the smoke-exposed mice shows that smoke was inhaled and reached the bowel, and the high cotinine levels (median 65 μg/l) indicate active exposure to CS, since in humans heavy exposure to passive smoking rarely results in cotinine levels >10 μg/l.[44] Another limitation is that we used for qPCR and western blotting in mice the whole colon, including the muscle layer, instead of a scraping of the mucosa. This could have caused a dilution of HO-1 expression.

In conclusion, although smoking induced HO-1 expression in colonic cells *in vitro*, no effect of smoking on *in vivo* colonic HO-1 expression in humans and mice was shown. So smoking does not exert its beneficial effect on UC via up-regulation of colonic HO-1. However, smoking could exert its beneficial effects on UC through an up-regulation of HO-1 in the lungs causing increased carboxyhemoglobulin levels. CS does induce colonic CYP1A1 expression which indicates that CS is able to trigger other protective mechanisms locally in the colon.

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Chapter 7

Smoking behaviour in liver transplant recipients

F. van der Heide, G. Dijkstra, R.J. Porte,
J.H. Kleibeuker, E.B. Haagsma

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Abstract

Introduction:
Long-term morbidity and survival after orthotopic liver transplantation (OLT) are for a main part determined by cardiovascular disease and cancer. Tobacco use is a well-known risk factor for both. The aim of this study was to examine smoking behaviour before and after OLT, and to define groups at risk for resuming tobacco use after OLT. In addition, we looked for a relation between smoking and morbidity after OLT.

Methods:
All 401 adult patients with a follow-up of at least 2 years after OLT were included. Data were collected from the charts. A questionnaire about smoking habits at four time points before and after OLT was sent to all 326 patients alive, and 301 (92%) patients responded.

Results:
Both before and after OLT, 53% of patients never used tobacco and around 17% were active smokers. Of the active smokers during the evaluation for OLT almost one third succeeded in cessation, often during the waiting time for OLT. Twelve percent of former smokers restarted smoking, mainly after OLT. Tobacco use was the highest in patients with alcoholic liver disease (52% active smokers before OLT, 44% after OLT), and the lowest in primary sclerosing cholangitis (1.4% active smokers before OLT). At 10 years the cumulative rate of malignancies was 12.7% in active smokers compared to 2.1% in non-smokers ($p=0.019$). No effect on skin cancer or cardiovascular disease was found.

Conclusion:
Smoking is a serious problem after OLT and increases the risk for malignancy. Prevention programs should not only focus on active smokers, but also on former smokers.

Introduction

Orthotopic liver transplantation (OLT) is an accepted therapy for end-stage liver disease. The last decades short term survival after transplantation has improved with nowadays a one year survival rate of 85% and a five year survival of 75% (http://www.optn.org). Besides recurrence of the primary disease, the main causes of death in liver recipients on the long term are cardiovascular events and malignancies.[1-6] Therefore it becomes more and more important to prevent diseases and health problems that are not associated with the original liver disease. Smoking is an important risk factor for cardiovascular events as well as for several malignancies. Smoking is considered one of the leading causes of preventable death in the general population and could also be an important one in liver transplant recipients. However, studies about the smoking behaviour of liver transplant recipients are scarce. Di-Martini et al. have prospectively studied the smoking behaviour in patients transplanted for alcoholic liver disease (ALD) and they found that recipients resume smoking early after OLT and increase consumption over time.[7] Ehlers et al. reported 15% active smokers after OLT and a relapse rate of 20% in former smokers.[8] Both studies were performed in the United States.

Studies on the effects of smoking on the long-term course after OLT are also scarce. Some studies have reported a possible relation between smoking and malignancies after OLT,[9,10] especially in recipients with ALD.[11-13] In a previous study from our group, a relation between smoking and cardiovascular events was suggested in a group of 331 OLT recipients.[6] In recipients of other solid organ transplants much more studies on the effects of smoking have been performed. These showed that tobacco use was associated with graft loss and mortality in renal transplant patients,[14,15] graft loss in pancreas transplant patients,[16] cardiovascular disease in renal transplant patients,[14,17-19] and malignancies in renal,[20] lung,[21] and heart transplant patients.[22]

The primary aim of the present study was to examine smoking behaviour before and after OLT, and to look for patient groups at risk for resuming smoking after OLT. In addition we looked for a relation between smoking and survival and morbidity after OLT.

Methods

Patients

All adult patients who had undergone liver transplantation in our hospital between 1979 and May 2005, and had a follow-up of at least two years after transplantation were included. Patient characteristics, pretransplant malignancies (including skin) and cardiovascular status, and the outcome variables were obtained through retrospective analysis of the medical charts. The immunosuppression protocol has been described before.[23,24] Briefly, patients transplanted between 1979 and 1986 received azathioprine and steroids. After 1986 cyclosporine was added, and after 1997 patients received a calcineurin-inhibitor (cyclosporine or tacrolimus) and steroids, with or without azathioprine. Some patients used sirolimus because of renal dysfunction.

Smoking behaviour

Data on smoking behaviour were collected in two ways. First, retrospectively from the medical charts, and second, by a questionnaire. From the charts we collected data at four time points: during evaluation for OLT (before listing), shortly before OLT, 2 years after OLT, and at the end of follow-up. At each time point it was determined if a patient was an active smoker, a former smoker, or a never smoker. A questionnaire was sent to all patients presently alive. In this questionnaire the patients were asked about their smoking habits at the four time points mentioned above. Of each time point it was asked if the patient was an active smoker, a former smoker, or a never smoker. In addition, questions were included on number of years of tobacco use, and, in case of a former smoker, when tobacco use was stopped, on type of tobacco product, on average number of cigarettes or otherwise per day, and on plans for smoking cessation and willingness to participate in a free smoking cessation course in our hospital. Smokers were defined as smoking seven or more cigarettes per week. For converting cigars to cigarettes we equated one cigar with four cigarettes, considering that an average cigar contains four grams and one cigarette one gram of tobacco.[25]

Pretransplant cardiovascular status and outcome variables

For pretransplant cardiovascular status we recorded treatment for diabetes mellitus and hypertension, and vascular events (angina pectoris, myocardial infarction, coronary interventions, transient ischemic attack, cerebral vascular accident, intermittent claudication and intervention for large vessel disease).

For outcome variables we looked at patient and graft survival, occurrence of hepatic artery thrombosis, *de novo* malignancies and *de novo* vascular events. For graft survival we recorded need and reason for retransplantation. Date was noted of all events and events were recorded till the end of follow-up, May 2007.

Ethical considerations

The methods used were discussed with the medical ethics committee. According to Dutch legislation there were no objections against the methods used. A returned questionnaire was considered as an informed consent.

Statistical analysis

Categorical variables are presented as frequencies with percentages and continuous variables as medians (range). The data were analyzed using the Statistical Package for the Social Sciences program version 14.0 (SPSS Inc., Chicago, Illinois, USA). Categorical variables were compared with the chi-square test and continuous variables with the Mann-Whitney test. Outcome variables were tested for significance by using the Kaplan-Meier method, with the log rank test. Differences are considered significant when $p \leq 0.05$.

Results

Clinical Characteristics

In our hospital 867 liver transplantations were performed between 1979 and May 2005. Of these, 526 were adult patients undergoing at least one liver transplant. Eleven patients

were lost to follow-up (6 living outside the Netherlands, 3 with follow-up elsewhere and 2 unknown) and of the remaining 515 recipients, 401 (78%) survived at least two years after transplantation and these were included in the present study. Clinical characteristics of the 401 recipients are shown in Table 1. At the time of this study 326 patients were alive and 75 were deceased. The most frequent indications for transplantation were primary sclerosing cholangitis (PSC) and primary biliary cirrhosis.

Smoking behaviour according to questionnaire

Three hundred and one of the 326 patients returned the questionnaire, a response rate of 92%. Next and in Figures 1-4 the results are presented at the time points evaluation for OLT, at time of OLT, 2 years after OLT, and at the end of follow-up (a median 8.6 (range 2-26) years after OLT).

Table 1. Patient characteristics

Characteristic	All patients (401)	Alive (326)	Deceased (75)
Female gender	208 (51.9)	164 (50.3)	44 (58.7)
Age at OLT (yrs, median, range)	46.5 (18.1-67.7)	45.7 (18.1-67.7)	50.3 (22.6-62.4)
Date of OLT (median, range)	Aug, 1997 (Apr, 1979-May, 2005)	Aug, 1998 (Apr, 1979-May, 2005)	Sep, 1993 (Oct, 1979-Nov, 2003)
Follow-up after OLT (yrs)			
(median, range)	8.6 (2.0-28.0)	8.7 (2.0-28.0)	7.3 (2.0-26.0)
(mean, SD)	9.3 (5.2)	9.6 (5.3)	7.9 (4.9)
Diagnosis (no of pts, %)			
Primary sclerosing cholangitis	78 (19.5)	71 (21.8)	7 (9.3)
Primary biliary cirrhosis	63 (15.7)	51 (15.6)	12 (16.0)
Viral (Hepatitis B and/or C)	46 (11.5)	37 (11.3)	9 (12.0)
Metabolic disease	45 (11.2)	39 (12.0)	6 (8.0)
Cryptogenic cirrhosis	39 (9.7)	29 (8.9)	10 (13.3)
Alcoholic cirrhosis	38 (9.5)	27 (8.3)	11 (14.7)
Autoimmune cirrhosis	34 (8.5)	24 (7.4)	10 (13.3)
Acute liver failure	20 (5.0)	19 (5.8)	1 (1.3)
Other	38 (9.5)	29 (8.9)	9 (12.0)
Malignancy in explant (no of pts, %)	35 (8.7)	24 (7.4)	11 (14.7)
Hepatocellular carcinoma	32 (8.0)	22 (6.7)	10 (13.3)
Cholangiocarcinoma	4 (1.0)	2 (0.6)	2 (2.7)
Immunosuppresion 1 year after OLT			
Cyclosporine/tacrolimus/sirolimus	229/134/9	178/125/8	51/9/1
Azathioprine/mycophenolate mofetil	269/32	208/26	61/6
Steroids	385	310	75
Immunosuppression end follow-up			
Cyclosporine/tacrolimus/sirolimus	117/112/7	86/103/6	31/9/1
Azathioprine/mycophenolate mofetil	246/36	191/29	55/7
Steroids	327	259	68

Abbreviations: OLT, orthotopic liver transplantation; SD, standard deviation.

Smoking behaviour

Both before and after OLT a majority of patients never smoked (53-54%). About a third of the patients were former smokers (28-32%) and a substantial minority of 14-18% was active smoker. In Figure 1 it is shown that at the several time points the percentages are rather stable, with only a small decline in active smokers shortly before OLT.

Smoking behaviour of never smokers. In Figure 2 it is shown that of the 163 patients who had never smoked at the time of evaluation, most refrained from smoking, with only 2.5% of the patients smoking at two years after OLT and 1.8% at the end of follow-up.

Smoking behaviour of former smokers. In Figure 2 it is shown that of the 85 patients who were former smokers at the time of evaluation, most refrained from smoking until OLT, but up to 12% restarted smoking after OLT. The 10 patients that relapsed after OLT had stopped smoking at a median of 10 months (1-36) before OLT. The 75 former smokers that did not relapse had stopped smoking 168 months (1-540) before OLT. The difference is statistically

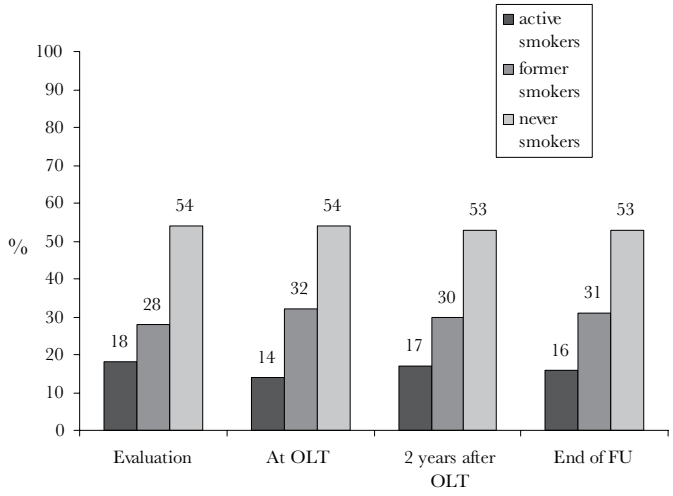

Figure 1. Smoking behaviour of the respondents to the questionnaire before and after OLT (n=301). Abbreviations: FU, follow-up; OLT, orthotopic liver transplantation.

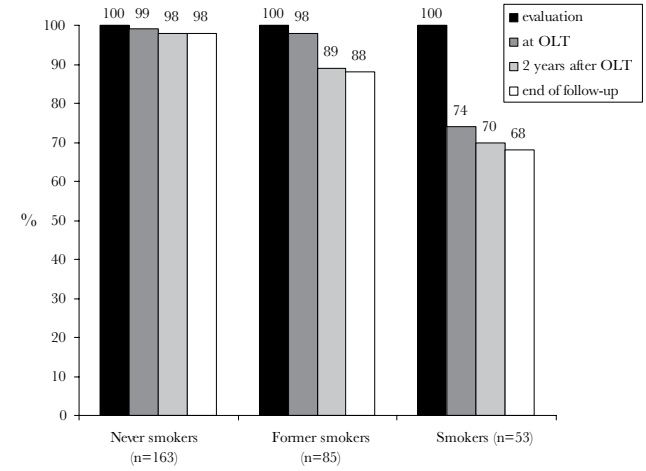

Figure 2. Course of smoking behaviour after evaluation for never smokers, former smokers and smokers. Abbreviation: OLT, orthotopic liver transplantation.

significant ($p<0.001$). Figure 3 shows in more detail that a minority of patients restarted smoking and later stopped again.

Smoking behaviour of active smokers. In Figure 2 it is shown that of the 53 active smokers at the time of evaluation, 26% succeeded in quitting of smoking before OLT, and this remained and even increased a little to 32% after OLT. But these are overall percentages. Figure 4 shows in more detail that a minority of these 53 patients first stopped, but later restarted smoking at some time after OLT, and sometimes stopped again later. At 2 years after OLT there were 50 active smokers, including (re)starters, and 20% succeeded in quitting smoking at the end of follow-up.

Relation between smoking and patient characteristics.

No relation was found between smoking and gender, and between smoking and age in smokers and never smokers. Former smokers at evaluation were older than never smokers

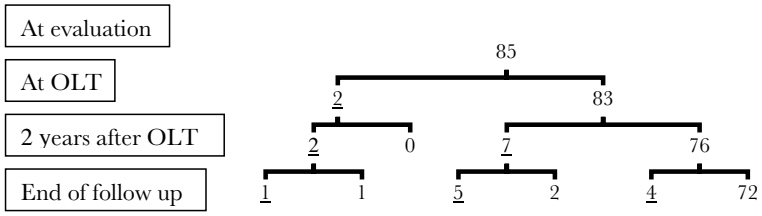

Figure 3. Former smokers at evaluation for OLT and changes in behaviour afterwards. The underlined numbers indicate the number of active smokers; the other numbers indicate the number of former smokers. Abbreviation: OLT, orthotopic liver transplantation.

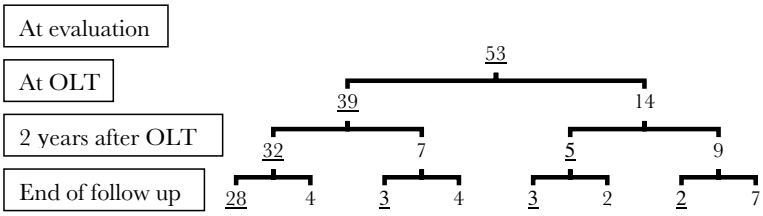

Figure 4. Active smokers at evaluation for OLT and changes in behaviour afterwards. The underlined numbers indicate the number of active smokers; the other numbers indicate the number of former smokers. Abbreviation: OLT, orthotopic liver transplantation.

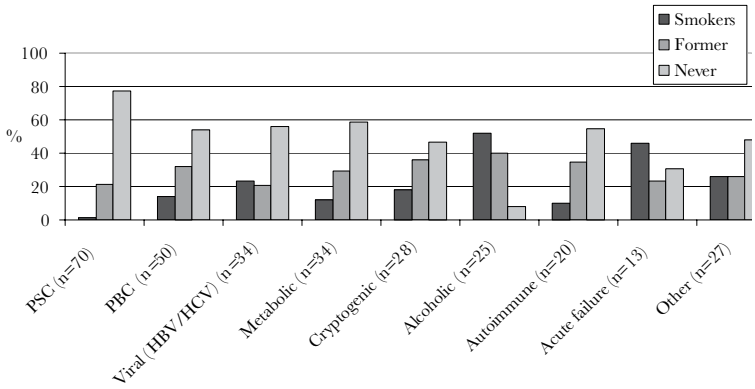

Figure 5. Smoking behaviour during evaluation for orthotopic liver transplantation according to primary liver disease. Abbreviations: HBV, Hepatitis B virus; HCV, Hepatitis C virus; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

(median 51.2 years vs 42.9 years; p=0.001) and active smokers (median 51.2 years vs 45.9 years; p=0.05). At the time of evaluation for OLT, more patients with ALD and with acute liver failure were active smokers (respectively 52 and 46%) compared to the other diagnoses (see Figure 5, p<0.001). In this respect the percentage of active smokers in the group with PSC was the lowest (1.4%). In the alcoholic group, only 8% were never smokers, whereas 77% of PSC patients had never smoked at the time of evaluation (Figure 5). At the end of follow-up the same pattern was seen with 44% active smokers in the alcoholic group, and only 4.3% in the PSC group (p<0.001). The number of active smokers (31%) in the acute liver failure group was not different anymore compared to the other diagnoses.

Smoking was also studied in ALD patients with respect to relapse into drinking. Of the 25 patients with alcoholic cirrhosis only six had one or more relapses into drinking after OLT, with one patient persistently drinking. Of the six relapsers, four were smokers at the end of follow-up (67%), one was a former smoker (17%) and one had never smoked (17%). Of the 19 nonrelapsers seven patients were smokers at the end of follow-up (37%), 11 were former smokers (58%) and one had never smoked (5%). There was a tendency for a lower number of former smokers in the relapsers compared to the nonrelapsers (p=0.078).

Smoking characteristics

Both before and after OLT, and in both active and former smokers, the large majority of patients smoked only cigarettes and/or hand-rolling tobacco (92% and 86% respectively). A minority preferred pipe and/or cigars (about 5%). No statistical differences between the different groups and time points were found in this respect.

At the end of follow-up, the then active smokers had smoked a median of 34 years (range 2-50), and they reported smoking a median of 11 cigarettes per day (range 2-60). At the end of follow-up the then former smokers had smoked a median of 15 years (range 3-45), and they reported smoking a median of 10 cigarettes per day (range 1-45). The former smokers had stopped a median of 16.5 years (range 0.1-46) before the end of follow-up.

Table 2. Tobacco use from medical charts versus questionnaires

Characteristic: n (%)	Medical charts	Questionnaire
Patients	301	301
Active smokers:		
Evaluation	44 (14.6)	53 (17.6)
At OLT	29 (9.6)	42 (14.0)
2 years after OLT	33 (11.0)	50 (16.6)
End of follow-up	40 (13.3)	49 (16.3)
Ever smoking		
Ever	101 (33.6)	142 (47.2)
Pre OLT	93 (30.9)	139 (46.2)
After OLT	67 (22.3)	65 (21.6)

Abbreviation. OLT, orthotopic liver transplantation.

Smoking cessation plans

In the questionnaire, the still smoking patients were asked about plans to quit smoking. Five of the 49 patients did not answer this item. Of the other 44 patients, 20 patients (45%) wanted to stop within six months, 7 (16%) within five years, 10 (23%) ever and 7 (16%) never. When offered to join a cost-free smoking cessation course, four of the 49 patients did not answer this item. Of the other 45 patients, 6 patients accepted the offer (13%), 28 (62%) refused and 11 (24%) were in doubt. For the intenders to stop smoking within 6 months, 30% accepted the offer.

Relation between smoking and outcome after liver transplantation

Relation to patient and graft survival

For the analysis of the effect of smoking on patient and graft survival, we first had to make a comparison between smoking behaviour data acquired from the charts and those acquired from the questionnaires. The charts turned out to be incomplete in detail. Comparison could be made however with respect to active smoking at the different time points before and after transplantation, and with respect to ever having smoked before or after liver transplantation. As is shown in Table 2 active smoking is underestimated in the charts, with up to one third less active smokers in the charts compared to the questionnaires. The same was true for ever smoking data, except for the time after OLT. In both the charts and the questionnaires 22% of patients ever smoked after OLT.

Based on this analysis, patient and graft survival were studied comparing patients of whom the charts mentioned smoking in the post-transplant period versus those of whom the charts mentioned no smoking or unknown. In the group of 401 patients no differences were found for patient and graft survival between the 103 ever smokers after OLT and the other 298 patients. Also after exclusion of the 59 patients with unknown behaviour, there were no differences in patient and graft survival between ever smokers and nonsmokers. Furthermore, no differences were found for the causes of graft failure between smokers and the other patients. Overall patient survival at one year was 100% (inclusion criterion), at five years 93%, at ten years 85%, at fifteen years 72% and at twenty years 64%. Overall graft survival at one year was 93%, at five years 82%, at ten years 73%, at fifteen years 60% and at twenty years 49%.

Table 3. Outcome for posttransplant active smokers and nonsmokers

Characteristic: n (%)	Smokers (n = 59)	Never smokers (n = 236)
Retransplantation	11 (18.6)	28 (11.9)
Hepatic artery thrombosis	9 (15.3)	17 (7.2)*
Cardiovascular events *de novo*	6/58 (10.3)	19/227 (8.4)
Hypertension *de novo*	25/54 (46.3)	113/220 (51.4)
Diabetes mellitus *de novo*	13/58 (22.4)	34/215 (15.8)
Malignancies (including skin) *de novo*	14/55 (25.5)	38/223 (17.0)
Malignancies (excluding skin) *de novo*	7/57 (12.3)	7/225 (3.1)**
Skin malignancies *de novo*	9/57 (15.8)	33/232 (14.2)

* p = 0.078; ** p = 0.019.

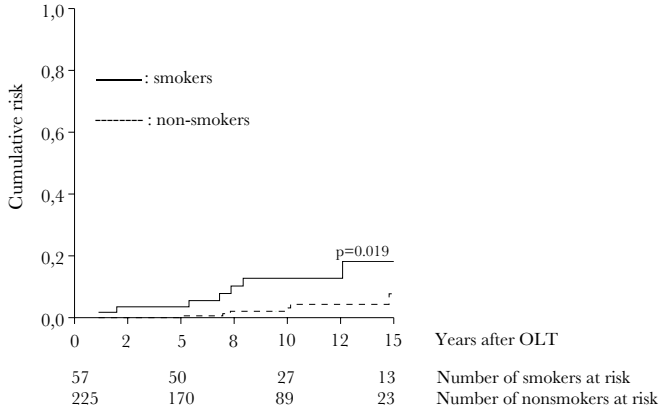

Figure 6. Cumulative risk of *de novo* nonskin malignancies between smokers and nonsmokers after OLT. Abbreviation: OLT, orthotopic liver transplantation.

Relation to morbidity

Because of lack of detail in the charts with respect to smoking behaviour, we decided to study the possible relation between smoking and post-transplant morbidity only in the group of responders to the questionnaire. Fifty nine responders who reported tobacco use at two years after OLT and/or at the end of follow-up were compared with 236 responders who reported to have abstained from tobacco use after OLT. The comparison is shown in Table 3. *De novo* malignancy, excluding skin cancer, developed significantly more often in smokers compared to nonsmokers (see Figure 6; p=0.019, Kaplan-Meier log rank test). The cumulative risk (standard error in brackets) for development of *de novo* malignancy in smokers was 3.5 (2.4), 12.7 (4.9), and 18.2 (7.0)% at 5, 10, and 15 years after OLT, respectively. In nonsmokers this was 0.6 (0.6), 2.1 (1.2), and 7.7 (3.8)%, respectively. Tumor types in smokers were oropharynx cancer (1 patient), cancer of the vulva or cervix (2 patients), colon cancer (2 patients), and post-transplant lymphoproliferative disorder (2 patients). Tumor types in nonsmokers were gastric, colon, and prostate cancer (1 patient each), post-transplant lymphoproliferative disorder (2 patients), and *de novo* hepatocellular carcinoma (1 patient). We found a tendency for hepatic artery thrombosis occurring more often in smokers compared to nonsmokers (p=0.078, Kaplan-Meier log rank test).

Discussion

Long-term morbidity and survival after OLT are determined for a main part by the development of cardiovascular diseases and cancer. Tobacco use is a well-known risk factor for both disease entities. In our country tobacco use is increasingly forbidden in public areas, but until July 1, 2008, smoking was still allowed in restaurants and bars. In our center discontinuation of tobacco use is encouraged before the actual transplant takes place, but is not a contraindication. The present study gives us insight into the smoking patterns and some negative effects of smoking. In addition, the study helps to recognize subgroups of patients who could be offered extra help in the future.

As we were mainly concerned with long term negative effects of smoking, we studied patients who survived at least 2 years after OLT. Detailed data were not available in the medical charts; therefore we used a questionnaire which was sent to all patients alive. The response rate was high (92%). Although this method has several drawbacks, including a recall bias of events that took place in the past, the facts that the questionnaires remained anonymous to the treating physicians and were sent several years after sofar successful transplantation must have helped the patients to report the truth.

Both before and after OLT a small majority of patients had never used tobacco and around 17% of the patients were active smokers. This figure compares favourable with the Dutch population as in 2007 28% of the Dutch population were reported to be active smokers (Dutch central agency for statistics; http://statline.cbs.nl). The percentage active smokers in a German population of kidney transplants was 13%,[26] and in Italian heart transplants 12%.[27] A positive finding was that of the patients that smoked at the time of the evaluation for OLT, almost one third succeeded in smoking cessation, often during the waiting time for OLT, although sometimes with ups and downs (Figures 2 and 4). That the overall percentage of active smokers before and after OLT were about the same implies that other patients, mainly former smokers at the time of evaluation (12%), restarted smoking after OLT (Figures 2 and 3). We found that especially former smokers who had succeeded in stopping tobacco use a relatively short time ago were at risk for a relapse.

To the best of our knowledge, there is only one study in liver transplant patients reported in the literature that is comparable to our study. Ehlers et al. studied patients transplanted in Florida.[8] Their method was a structured interview by telephone with a 42% response rate. They reported 15% active smokers after OLT and a relapse rate of 20% in former smokers. Both figures are comparable with our findings. However, the response rate of 42% makes the possibility of an underestimation higher than in our study with a response rate of 92%, and therefore comparison of the data remains problematic. From both studies it follows that intervention programs should not only be aimed at active smokers, but also at former smokers, and that these programs should be continued for many years, if not life long.

We found that most former smokers started smoking early in their life and quitted after a median 15 years of smoking, long before transplantation. The range was wide however. At the end of follow-up the cumulative number of smoking years was twice as high in the active smokers compared to the former smokers. It was no surprise that almost all patients used tobacco by smoking cigarettes and/or hand-rolling tobacco. Pipes or cigars were enjoyed by only 5% of the patients.

Tobacco use was highest in patients with ALD and in those with acute liver failure. During evaluation 52% of the alcoholic group were active smokers (and only 8% never smokers).

The combination of alcohol and tobacco addiction is well known and has been reported to be as high as 90% in alcohol abusers.[28,29] This combination is also shown in our finding of a tendency for a higher number of former smokers in ALD patients without a relapse into drinking, which suggests that nonrelapsers are more likely to stop smoking than relapsers. As alcohol abstinence for at least six months was a prerequisite for OLT in our center, it is disturbing that still so many continued active smoking. At the end of follow-up active smoking in this group had decreased from 52% to 44%; this decrease is remarkable when compared to the whole group (18% to 16%). Probably there is already more attention for this group of patients or attention to stop alcohol use also influences smoking behaviour, but clearly more is needed to further bring down these figures. DiMartini et al. from Pittsburgh mentioned tobacco use after OLT for alcoholic liver disease an underestimated problem as these patients were reported to have a high incidence of lung and pharyngeal cancer.[7,30-32] They found that on average more than 40% of patients with ALD were smoking after OLT and that smoking was resumed already at 3 months post-OLT. Our data seem comparable, except that we show an overall decrease if reckoned from evaluation for OLT onwards.[7]

Remarkable is the PSC group with only 1.4% active smokers and 77% never smokers at the time of evaluation for OLT. A possible protective effect of smoking on the development of PSC[33-36] and ulcerative colitis[37-39] has been shown before. The exact protective mechanism of smoking is not yet known. Studies with nicotine as therapy for PSC showed no beneficial effects.[40,41] It would be interesting to study the effects of smoking on recurrent PSC after OLT, but because of the low number of tobacco users in this patient group, this will only be possible in a multicenter study group.

We did not find a negative effect of active smoking after OLT on long-term patient and graft survival sofar, but this item could be studied only with smoking data from the medical charts, and these data were incomplete. However, in the group of survivors who responded to the questionnaire, we did find that the prevalence of nonskin cancer was significantly increased in active smokers after OLT. At ten years the cumulative malignancy rate was 12.7% in smokers compared to 2.1% in non-smokers after OLT. Malignancies seemed to develop much earlier after OLT in tobacco users (Figure 6). We did not notice a difference in types of tumors that developed. Also no effect on skin cancer was found. Tobacco use as a risk factor for malignancy after OLT has been recognized before.[9-13] Previous studies however relied on data in medical charts and most often not on actual posttransplant smoking data.

We did not find an increased prevalence of cardiovascular disease in smokers. Most likely tobacco use is only one of the many risk factors for cardiovascular disease after OLT, such as overweight, diabetes, hypertension, and the use of immunosuppressive agents.

In summary, both before and after OLT about 17% of patients are active smokers, but after OLT this percentage includes restarting former smokers and a decreasing number of actively smoking ALD patients. Proportionally, most tobacco users are found in the ALD group, and least in the PSC group. Pretransplant intervention programs should be aimed not only on pretransplant active smokers, but also on former smokers, especially those who stopped smoking quite recently. The higher prevalence of malignancies in active smokers after OLT warrants intervention programs after OLT and regular screening for malignancies.

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Chapter 8

Summary and future perspectives

Summary

Two important groups of patients for the gastroenterologist and hepatologist are patients with inflammatory bowel diseases (IBDs) and liver diseases. The two most common IBDs are Crohn's disease (CD) and ulcerative colitis (UC), and they are characterized by relapsing inflammation of the gastrointestinal tract. In CD the entire gastrointestinal tract can be involved, while in UC the inflammation is limited to the colon. Another remarkable difference between CD and UC is the opposite effect of smoking. Smoking seems detrimental for CD, but beneficial for UC. Probably due to this remarkable opposite effect, smoking is a widely studied factor in IBD. The role of active smoking on the development of IBD is clear; smoking is a risk factor for developing CD, but protective for UC. The role of passive smoking on the development of IBD is not clear. Studies on the effects of active smoking on the disease course of CD and UC are inconclusive; several studies showed detrimental effects on the course of CD and beneficial effects on UC, but for both diseases other studies could not confirm this. Finally, the role of passive smoking on the course of CD and UC is unknown.

It is unknown why smoking is detrimental for CD and beneficial for UC. Mechanisms that could be involved are the Toll-like receptor-4-dependent pathway in macrophages, the heme-oxygenase-1 (HO-1) pathway and thrombogenic effects of tobacco on the intestinal microvasculature. The understanding of the mechanism is complicated by the large number of components in cigarette smoke. Nicotine and carbon monoxide are the most studied components, and both have detrimental and beneficial effects on intestinal inflammation.

The other important group is patients with liver diseases. Orthotopic liver transplantation (OLT) is an accepted therapy for end-stage liver disease. The prognosis of recipients has increased over the last decades. The most important causes of morbidity and mortality after OLT are malignancies and cardiovascular diseases. Smoking is a notorious risk factor for several malignancies and cardiovascular diseases in the general population, but studies about the role of smoking in recipients of liver transplants are scarce. The few reports available about the effects of smoking on OLT recipients show that smoking is a risk factor for several complications and serious events after OLT. However, literature on smoking behaviour in recipients is disappointingly scarce.

This thesis focuses on the role of active and passive smoking in IBD, and on the role of smoking in OLT recipients.

Chapter 1 starts with an introduction on the role of smoking in IBD and in OLT recipients, and describes the aims and outlines of the thesis.

In view of the opposite effect of smoking on CD and UC, different changes in smoking behaviour after diagnosis between CD and UC patients are likely. In chapter 2 we studied changes in active smoking, cessation plans and passive smoking in 380 CD and 295 UC patients using a written questionnaire. More ever smoking UC patients stopped smoking before diagnosis than CD patients (63 vs. 22%; $p<0.001$), resulting in 30% former smokers at diagnosis in UC and 13% in CD ($p<0.001$). The smoking cessation rates at and after diagnosis are equal between CD and UC. Half of the CD patients stopped smoking after diagnosis leading to fewer present smokers in CD than in a control population (26 (95% confidence interval: 21–30%) vs. 33%). For both CD (22 vs. 35%; $p=0.044$) and UC (24 vs. 53%; $p=0.024$) continuing smokers after diagnosis were less often highly educated than

quitters. Cessation plans (89%), passive smoking in childhood and present passive smoking were not different between CD and UC patients. We conclude that there are no differences in changes in smoking behaviour at and after diagnosis between CD and UC patients, suggesting a lack of knowledge by these patients about the link between their disease and smoking behaviour. However, CD patients seem less refractory to smoking cessation than the general population considering that half of the CD patients stopped smoking after diagnosis. Therefore it is worthwhile putting energy in helping CD patients stop smoking. In both CD and UC, a higher education is associated with smoking cessation after diagnosis.

Studies about the role of smoking on the disease course of CD as well as UC give conflicting results, and studies about the effect of passive smoking on the course of IBD are rare or non-existing. In chapter 3 we retrospectively studied the effects of active and passive smoking in 380 CD and 295 UC patients from a university hospital cohort. Smoking behaviour was defined by using the same questionnaire as in chapter 2, and data on the disease course was obtained from patient records. At diagnosis there were 52% smokers in CD, 41% in the general population, and 28% in UC. The number of present smokers in CD is lower than the present number of smokers in the general population (26 vs. 35%). No detrimental effects of active smoking on CD were observed, but passive smokers needed more frequently immunosuppressants and infliximab than non-passive smokers. Active smoking had beneficial effects on UC, indicated by reduced rates of colectomy, primary sclerosing cholangitis and backwash-ileitis in active smokers compared to never smokers, and higher daily cigarette dose correlated with less extensive colitis and a lower need for therapy. Furthermore, smoking cessation after diagnosis was detrimental for UC patients, indicated by increased needs for steroids and hospitalizations for patients that stopped smoking after diagnosis compared to before diagnosis. The conclusion of this study is that active smoking is a risk factor for CD, but does not affect the disease course. Passive smoking is detrimental for the outcome of CD patients. In UC, active smoking shows dose-dependent beneficial effects on the outcome. Our data suggest that passive smoking is a novel risk factor for CD.

The results of our study in chapter 3 were somewhat unexpected, mainly because the detrimental effect of active smoking on CD was not confirmed, but also because of the finding of opposite effects of active and passive smoking. Some of this could be caused by studying patients from a university hospital with an important referral function. Studying patients referred from other hospitals causes a selection bias, since patients with a more benign disease course could be underrepresented. The effects of smoking on the disease course could be so subtle, that they are more pronounced in patients with a benign disease course. In chapter 4 we aimed to see whether the findings in chapter 3 could be confirmed in an IBD cohort from a regional hospital. We retrospectively studied the effects of active and passive smoking in 128 CD and 192 UC patients from a regional hospital. We used the same methods as in chapter 3. At diagnosis there were 52% (95% confidence interval: 43–60%) smokers among CD patients, 40% in a control population and 25% (95% confidence interval: 18–31%) among UC patients. There were less former (19 vs. 31%, $p=0.013$) and never smokers at diagnosis (30 vs. 44%, $p=0.009$) in CD than in UC. No detrimental effects of active or passive smoking on the course of CD were observed. UC patients who continued smoking after diagnosis needed less often two or more hospitalizations than never smokers (5 vs. 25%, $p=0.036$). Otherwise no clear beneficial effects of active smoking on UC were observed. Passively smoking UC patients experienced more often extraintestinal manifestations (25 vs. 7%, $p=0.029$) than non-passive smokers. We conclude that also in an IBD population from

a regional hospital smoking is a risk factor to develop CD and protects against developing UC. We found no detrimental effects of smoking on the disease course of CD and no clear beneficial effects on the course of UC.

Besides smoking behaviour, another important factor for the development of CD is the genetic background. During the last decade a still increasing number of genetic variants have been found to be associated with IBD, especially with CD. It is likely that the development of CD is partly caused by an interaction between these genetic variants and smoking, but studies investigating this interaction are scarce. In chapter 5 we studied differences in disease associated genetic variants between CD patients stratified for active smoking at diagnosis and for passive smoking in childhood. We selected 19 confirmed genetic variants located in 14 CD-associated genes or loci, including three *NOD2* variants. Genotyping data of these 19 CD-associated so-called single-nucleotide polymorphisms (SNPs) were available for 310 CD patients and 976 controls from previous studies by our group. We studied the allelic associations of these 19 CD-associated SNPs in CD patients stratified for active smoking at diagnosis and passive smoking in childhood. Data on active smoking at diagnosis and passive smoking in childhood were obtained through the previously mentioned written questionnaire and review of the medical charts. The loci associated in smoking, but not in non-smoking, CD patients were 5p13.1 (rs17234657), *DLG5* (rs2165047), *NKX2-3* (rs10883365) and *NOD2* (R702W). The loci associated in non-smoking, but not in smoking, CD patients were *IL23R* (rs7517847), 5p13.1 (rs9292777), *IRGM* (rs13361189 and rs4958847), *IL12B* (rs6887695) and *CCNY* (rs3936503). *PTPN2* (rs2542151) was only associated in the smoking CD cohort ($p=0.04$), and not in the entire cohort ($p=0.23$) or in the non-smoking CD cohort ($p=0.80$). In passively smoking CD patients, associations with 13 SNPs in 9 loci were found, including *PTPN2*. In non-passively smoking CD patients, only associations with *NOD2* (1007fsinsC and G908R) were found. In conclusion, we found different genetic associations in different groups according to active smoking at diagnosis and passive smoking in childhood. A part of these differences can be explained by the lack of power in this relatively small CD cohort. However, the difference in associated genes between smoking and non-smoking CD patients implies a complex gene–environment interaction. Therefore, genetic studies of CD should be stratified for smoking behaviour, as otherwise moderately associated genes such as *PTPN2* can be missed.

It is unknown why smoking is beneficial for UC. One of the pathways involved in the beneficial effects could be the heme-oxygenase 1 (HO-1) pathway. HO-1 is the rate-limiting enzyme involved in the breakdown of heme, yielding the end-products biliverdin, Fe^{2+} and carbon monoxide. Heme causes oxidative stress, while all three end-products have anti-oxidative, anti-apoptotic and anti-inflammatory properties. Induction of HO-1 was beneficial in animal models of intestinal injury. Cigarette smoke (CS) is able to induce HO-1 in several human cells, but the effect of smoking on colonic HO-1 expression is unknown. Induction of colonic HO-1 by smoking could be beneficial for UC patients by protecting against development of UC and/or by ameliorating inflammation in established UC. In chapter 6 we performed a laboratory study on the effects of smoking on the colonic expression of HO-1 *in vitro*, in animals and in humans. For the *in vitro* part DLD-1 cells were incubated with cigarette smoke extract (CSE), for the animal part mice were exposed to CS, and for the human part colon biopsies of healthy human non-smokers and smokers were collected. HO-1 expression was evaluated by quantitative polymerase chain reaction (qPCR), western blotting and immunohistochemistry. Cytochrome P450 1A1 (CYP1A1) expression (qPCR) was

used as a positive control for exposure of the colon to CS. CSE dose-dependently induced HO-1 and CYP1A1 expression in DLD-1 cells. In mice and humans, colonic CYP1A1 mRNA expression was increased in subjects exposed to CS, but colonic HO-1 mRNA and protein expression were equal between controls and subjects exposed to CS. In the humans smoking exposure was in the therapeutic range for UC. The conclusion of this study is that CS does induce a CYP1A1 response, but not a HO-1 stress response in colonic epithelial cells. This indicates that CS does not exert its beneficial effect on UC via up-regulation of colonic HO-1, but could trigger other protective mechanisms locally in the colon.

Literature about the role of smoking in recipients of liver transplants is scarce. The few reports available about the effects of smoking on OLT recipients show that smoking is a risk factor for several complications and serious events after OLT. Considering this detrimental effect of smoking after OLT, it is disappointing that only two transplant centers have studied the smoking behaviour in OLT recipients. In chapter 7 we report about the smoking behaviour of OLT recipients before and after transplantation, and about the association of smoking and the occurrence of malignancies and cardiovascular diseases after OLT. One of the aims was to define groups at risk for resuming smoking after OLT. All 401 adult patients with a follow-up of at least 2 years after OLT were included. Data were collected from the medical charts. A questionnaire about smoking habits at 4 time points before and after OLT was sent to all 326 patients that were alive, and 301 (92%) patients responded. Both before and after OLT, 53% of patients never used tobacco and around 17% were active smokers. In 2007 28% of the Dutch population were active smokers. Of the active smokers during the evaluation for OLT, almost one-third succeeded in cessation, often during the waiting time for OLT. Twelve percent of former smokers restarted smoking, mainly after OLT. Especially former smokers who had succeeded in stopping tobacco use a relatively short time ago were at risk of relapse. Tobacco use was the highest in patients with alcoholic liver disease (52% were active smokers before OLT, and 44% after OLT) and the lowest in patients with primary sclerosing cholangitis (1.4% were active smokers before OLT). At 10 years, the cumulative rate of malignancies was 12.7% in active smokers versus 2.1% in non-smokers (P=0.019). No association with skin cancer or cardiovascular disease was found. In conclusion, before and after OLT about 17% of patients are active smokers. After OLT this percentage includes restarting former smokers and a decreasing number of active smokers transplanted for alcoholic liver disease. Prevention programs should focus not only at active smokers, but also at former smokers, especially those who stopped smoking quite recently. The higher prevalence of malignancies in active smokers after OLT warrants intervention programs after OLT and regular screening for malignancies.

Future perspectives

Despite the enormous amount of studies on the effects of smoking on the disease course of CD and UC, it is still not clear which role smoking plays in the course of both diseases. One of the findings of this thesis is that we found no detrimental effect of smoking on the course of CD. This is not in line with some studies that found a clear detrimental effect. Probably, smoking is only detrimental for the course of CD for selected patients. The susceptibility of

patients could depend on gender, disease location and disease severity. For example, it is suggested that especially female CD patients are affected by smoking. In this thesis we could not confirm this. Future studies should focus on selecting patient groups that are susceptible to the effects of smoking. We were the first to study the effects of passive smoking on the course of IBD. We studied this in a population from a university hospital and a regional hospital. We found a detrimental effect of passive smoking on CD patients from the university hospital, but could not confirm this in the regional hospital population. We need more studies on the effects of passive smoking on the course of IBD to determine the role of passive smoking.

In this thesis we have shown that the development of CD depends on an interaction between smoking and genetics. It is likely that the effects of smoking on the disease course also depend on the genetic background. Hopefully, future genetic studies are able to select genetic variants that make patients susceptible to the effects of smoking. At this moment the course of CD is unpredictable, but this might change in the near future. Members of our group showed that it might be possible to identify subgroups of CD patients with a severe prognosis based on the genetic variants. They found that an increasing number of risk alleles is associated with a more severe course of the disease.[1] Future studies should aim to classify CD patients based on a combination of the genetic background and smoking behaviour. Hopefully, this gives the opportunity to identify specific patient groups and to adjust treatment; some patients might benefit from early intervention and aggressive therapy, and other patients could be spared the side effects of unnecessary therapy.

From the findings from our study on the interaction between smoking and genetics, it is clear that specific environmental factors are necessary for specific genetic variants to contribute to disease development. This is an important finding, as most genetic case – control studies are not stratified for environmental factors and are not matched with data on environmental factors in control cohorts. In the future, we maybe able to find more CD-associated genes, and probably also UC-associated genes, by stratifying genetic studies for smoking behaviour and/or other environmental factors. This will be particularly true for associated genes with low odds ratios.

Although the effects of smoking on the development and course of IBD are already studied for decades, it is still unknown which pathways are influenced by smoking and which component(s) of cigarette smoke is/are responsible. In this thesis we studied the heme-oxygenase 1 (HO-1) pathway. Though cigarette smoke induces HO-1 expression in *in vitro* experiments with DLD-1 cells, we found no effect of cigarette smoke on colonic up-regulation of HO-1 in mice and humans. However, this does not mean that the HO-1 pathway is not involved in the beneficial effect of smoking on UC, since smoking could exert its beneficial effects through an up-regulation of HO-1 in the lungs causing increased carboxyhemoglobulin levels. Several interventions studies in mice showed beneficial effects of carbon monoxide on intestinal injury.[2-4] Hopefully, in the future intervention studies with carbon monoxide will proof to be beneficial in UC patients. In this thesis we showed that cigarette smoke reaches the colon, since colonic CYP1A1 expression is increased in smokers. Therefore, cigarette smoke may trigger other protective mechanisms locally in the colon, and future studies are needed to find these protective mechanisms.

Considering the possible detrimental effects of smoking on CD, one of the therapeutic goals in CD must be smoking cessation. In this thesis we showed that half of the CD patients stopped smoking after diagnosis, and we concluded that CD patients seem less refractory

to smoking cessation than the general population. A French study showed that after intervention only 12% of smokers quit for more than 1 year, and only 10% achieved prolonged abstinence.[5] A recent study suggested that the high knowledge about the risks of smoking to health and the low nicotine dependence in CD patients suggests that most patients with CD could be helped to stop smoking successfully in the IBD clinic without specialist treatment.[6] In contrast to this study, another study showed that CD patients had a higher rate of the smoking-linked personality characteristic "impulsive sensation seeking" which is associated with a poor smoking cessation rate.[7] The authors of this study suggested that CD patients with this higher impulsive sensation seeking need specific strategies for smoking cessation. Future studies are needed to clarify if CD patients need special cessation programs and how these programs should look like.

Every gastroenterologist will agree that we should advise CD patients to stop smoking. However, the situation on this matter for UC patients is complicated. As long as we don't know which component in cigarette smoke causes the beneficial effect on UC, it remains difficult what we should advise UC patients. It is important that we should not discourage patients with UC from stopping smoking, since on the long run patients are protected against smoking-related diseases; patients with UC had a decreased risk of lung cancer [8,9] and cardiovascular disease [8,10] compared to patients with CD.

The second focus of this thesis is the role of smoking in OLT recipients. In patients who are under consideration for OLT, cessation of alcohol consumption is required to be eligible for OLT, especially for those with alcoholic liver disease. The question is whether smoking cessation should also be introduced as an eligibility criterion for OLT. We found in this thesis that before and after OLT about 17% of patients are active smokers, and that active smokers after OLT have a higher prevalence of malignancies. Considering other studies that also showed detrimental effects of smoking on the long course after OLT, smoking cessation should be a major goal after OLT. Future studies on the effects of smoking on acute complications after OLT, like hepatic artery thrombosis [11] and transplant failure, can answer the question whether smoking cessation should also be a major goal before OLT. Interesting is a recent study that showed that active smokers had a 92% higher rate of biliary complications than lifetime non-smokers.[12] Considering this last study, and the remarkable low percentage of 1.4% active smokers in the primary sclerosing cholangitis group as shown in this thesis, it would be interesting to study the effects of smoking on recurrent primary sclerosing cholangitis after OLT. However, because of the low number of tobacco users in this patient group, this will only be possible in a multicenter study group.

Concluding, studies provided us with a lot of knowledge on the effects of smoking on CD and UC, including the studies in this thesis. However, the true role of smoking on CD and UC is still not clear, and more important, we still do not know which mechanisms are involved by smoking. The last decade a still increasing number of genetic variants have been found to be associated with IBD. In thesis we showed an interaction between these genetic variants and smoking on the development of CD. These findings proof that future genetic studies on CD and UC should be stratified for smoking behaviour. In OLT recipients cardiovascular diseases and malignancies are important causes of morbidity and mortality. In this thesis we showed a high and constant number of smokers before and after OLT, and that smoking is associated with malignancies after OLT. These results warrant intervention programs for smoking cessation and regular screening for malignancies in OLT recipients.

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Chapter 9

Nederlandse samenvatting en toekomstperspectieven

Samenvatting

Twee belangrijke groepen patiënten voor de maag-darm-leverarts zijn patiënten met chronische inflammatoire darmziekten (inflammatory bowel diseases – IBD) en met leverziekten. De twee belangrijkste chronische inflammatoire darmziekten zijn de ziekte van Crohn (ZvC) en colitis ulcerosa (CU); beide worden gekenmerkt door terugkerende episodes van ontsteking van het maagdarmkanaal. Bij de ZvC kan het gehele maagdarmkanaal zijn aangedaan, terwijl bij CU de ontsteking zich beperkt tot de dikke darm. Een ander opmerkelijk verschil tussen de ZvC en CU is het tegengestelde effect van roken; roken lijkt nadelig te zijn voor de ZvC, maar gunstig voor CU. Waarschijnlijk door dit opmerkelijk tegengestelde effect zijn er veel studies gedaan naar de rol van roken in IBD. De rol van actief roken op het ontwikkelen van IBD is duidelijk: het is een risicofactor voor het ontwikkelen van de ZvC, maar beschermt tegen CU. In tegenstelling tot de duidelijke rol van actief roken, is het onduidelijk wat de rol van passief roken op het ontwikkelen van IBD is. Verder zijn studies over de effecten van actief roken op het beloop van de ZvC en CU niet eenduidig. Meerdere studies toonden een nadelig effect van actief roken op het beloop van de ZvC en een gunstig effect op dat van CU, maar voor beide ziektes konden andere studies dit niet bevestigen. Ten slotte is de rol van passief roken op het beloop van de ZvC en CU onbekend.

Het is onbekend waarom roken nadelig is voor de ZvC en gunstig voor CU. Mogelijk betrokken mechanismen zijn de Toll-like receptor-4-afhankelijke pathway in macrofagen, de heme-oxygenase-1 (HO-1) pathway en trombogene effecten van tabak op de intestinale microvasculatuur. Het begrijpen van het mechanisme wordt gecompliceerd door de vele componenten in sigarettenrook. De meest bestudeerde componenten zijn nicotine en koolstofmonoxide, en beide hebben nadelige en gunstige effecten op darmontsteking.

Patiënten met leverziekten zijn een andere belangrijke groep. Levertransplantatie (LT) is een geaccepteerde behandeling voor eindstadium leverziekte. De prognose van de ontvangers van een LT is de laatste decennia verbeterd. De belangrijkste oorzaken van morbiditeit en mortaliteit na LT zijn maligniteiten en hart- en vaatziekten. Roken is een beruchte risicofactor voor verschillende maligniteiten en hart- en vaatziekten in de algemene populatie, maar studies over de rol van roken in ontvangers van een LT zijn schaars. De paar studies die er zijn over de effecten van roken na LT laten zien dat roken een risicofactor is voor verschillende complicaties en ernstige aandoeningen na LT. Helaas is er weinig bekend over het rookgedrag van ontvangers van een LT.

Dit proefschrift is gefocust op de rol van actief en passief roken in IBD, en op de rol van roken in ontvangers van een LT.

Hoofdstuk 1 begint met een introductie over de rol van roken bij mensen met IBD en ontvangers van een LT, en geeft een overzicht van de onderzoeksdoelen van dit proefschrift.

Gezien het tegengestelde effect van roken op de ZvC en CU, treden er na diagnose bij patiënten met de ZvC en CU waarschijnlijk verschillende veranderingen op in rookgedrag. In hoofdstuk 2 hebben we veranderingen in actief roken, stopplannen en passief roken bestudeerd in 380 patiënten met de ZvC en 295 met CU. Hiervoor gebruikten we een schriftelijke enquête. Van de ooit rokende UC patiënten stopten meer met roken voor de diagnose dan ZvC patiënten (63 vs. 22%; $p<0.001$), wat leidde tot 30% ex-rokers ten tijde van diagnose bij CU en 13% bij de ZvC ($p<0.001$). Het percentage dat stopte met roken ten tijde van en

na diagnose was gelijk voor de ZvC en CU. De helft van de ZvC patiënten stopte met roken na de diagnose wat leidde tot minder huidige rokers bij de ZvC dan in een controle populatie (26 (95% betrouwbaarheidsinterval: 21–30%) vs. 33%). In geval van zowel de ZvC (22 vs. 35%; p=0.044) als CU (24 vs. 53%; p=0.024) waren patiënten die na diagnose doorrookten minder vaak hoogopgeleid dan de stoppers. Stopplannen (89%), blootstelling aan sigarettenrook in de kindertijd en huidig passief roken waren niet verschillend tussen ZvC en CU patiënten. Wij concluderen dat er geen verschillen zijn in veranderingen in rookgedrag tussen ZvC en CU patiënten ten tijde van en na diagnose, wat suggereert dat deze patiënten niet goed op de hoogte zijn van de link tussen hun ziekte en rookgedrag. Toch lijken ZvC patiënten vatbaarder voor het stoppen met roken dan de algemene populatie aangezien de helft van de ZvC patiënten stopte met roken na diagnose. Dus is het geen verspilde energie ZvC patiënten te helpen met het stoppen met roken. Bij zowel ZvC als CU patiënten is een hogere opleiding geassocieerd met stoppen met roken na diagnose.

Studies over de rol van roken op het ziektebeloop van zowel de ZvC als CU laten tegenstrijdige resultaten zien, en studies over de effecten van passief roken op het beloop van IBD zijn zeldzaam of afwezig. In hoofdstuk 3 hebben we retrospectief de effecten van actief en passief roken bestudeerd in 380 patiënten met de ZvC en 295 met CU uit een academisch ziekenhuis cohort. Voor het definiëren van rookgedrag gebruikten we dezelfde enquête als in hoofdstuk 2, en data over het ziektebeloop werden verkregen door statusonderzoek. Ten tijde van diagnose rookte 52% van de ZvC patiënten, 41% in een controlepopulatie, en 28% van de CU patiënten. Het aantal huidige rokers bij de ZvC is lager dan in de algemene populatie (26 vs. 35%). Er werden geen nadelige effecten van actief roken op het beloop van de ZvC gezien, maar passieve rokers hadden vaker immuunsuppressiva en infliximab nodig dan patiënten zonder blootstelling aan passief roken. Actief roken had gunstige effecten op CU, wat bleek uit een lager aantal colectomieën, en minder vaak primaire scleroserende cholangitis en backwash-ileitis in actieve rokers dan in nooit-rokers. Daarnaast was een hoger aantal sigaretten per dag gecorreleerd met een minder uitgebreide ontsteking van de dikke darm en een geringere behoefte aan therapie. Stoppen met roken na diagnose was nadelig voor CU patiënten aangezien patiënten die na diagnose stopten vaker steroïden en opnames nodig hadden dan patiënten die voor diagnose stopten. De conclusie van deze studie is dat actief roken een risicofactor is voor het krijgen van de ZvC, maar het ziektebeloop niet beïnvloedt. Passief roken is nadelig voor het beloop van ZvC patiënten. Voor CU heeft actief roken dosisafhankelijke gunstige effecten op het beloop. Onze data suggereren dat passief roken een nieuwe risicofactor is voor de ZvC.

De resultaten van hoofdstuk 3 waren enigszins onverwacht, vooral doordat het nadelige effect van actief roken op de ZvC niet werd bevestigd, maar ook door de tegengestelde effecten van actief en passief roken. Deels zou dit veroorzaakt kunnen zijn door het bestuderen van patiënten uit een academisch ziekenhuis met een belangrijke referentiefunctie. Het bestuderen van patiënten die verwezen zijn vanuit andere ziekenhuizen kan leiden tot een selectiebias, want patiënten met een gunstiger ziektebeloop kunnen ondervertegenwoordigd zijn. De effecten van roken op het beloop kunnen zo subtiel zijn, dat zij meer uitgesproken zijn in patiënten met een gunstig beloop. Het doel van hoofdstuk 4 was om te zien of de bevindingen van hoofdstuk 3 bevestigd konden worden in een IBD cohort uit een regionaal ziekenhuis. We hebben retrospectief de effecten van actief en passief roken onderzocht in 128 patiënten met ZvC en 192 met CU uit een regionaal ziekenhuis. Hiervoor werden dezelfde methoden als in hoofdstuk 3 gebruikt. Ten tijde van diagnose rookte 52% (95%

betrouwbaarheidsinterval: 43–60%) van de ZvC patiënten, 40% in een controle populatie en 25% (95% betrouwbaarheidsinterval: 18–31%) van de CU patiënten. Er waren minder ex-rokers (19 vs. 31%, p=0.013) en nooit-rokers ten tijde van diagnose (30 vs. 44%, p=0.009) bij ZvC dan bij CU. Er werden geen nadelige effecten van actief en passief roken op de ZvC gezien. CU patiënten die doorrookten na diagnose hadden minder vaak twee of meer opnames nodig dan nooit rokers (5 vs. 25%, p=0.036). Voor de rest werden er geen duidelijke gunstige effecten van roken op CU gezien. Passief rokende CU patiënten hadden vaker extra-intestinale manifestaties (25 vs. 7%, p=0.029) dan patiënten zonder blootstelling aan passief roken. We concluderen dat ook in een IBD populatie van een regionaal ziekenhuis roken een risicofactor is voor het ontwikkelen van de ZvC en beschermt tegen CU. We vonden geen nadelige effecten van roken op het ziektebeloop van de ZvC en geen eenduidig gunstige effecten op het beloop van CU.

Naast rookgedrag is de genetische aanleg ook een andere belangrijke factor voor het ontwikkelen van de ZvC. Het laatste decennium is een nog steeds groeiend aantal genetische varianten gevonden die geassocieerd zijn met IBD en vooral de ZvC. Het is waarschijnlijk dat de ontwikkeling van de ZvC wordt beïnvloedt door een interactie tussen deze genetische varianten en roken, maar er zijn weinig studies die dit hebben onderzocht. In hoofdstuk 5 hebben we verschillen in ziektegeassocieerde genetische varianten onderzocht tussen ZvC patiënten gestratificeerd voor actief roken bij diagnose en voor passief roken in de kindertijd. We selecteerden 19 bevestigde genetische varianten in 14 ZvC-geassocieerde genen of loci inclusief drie *NOD2* varianten. Genotype data van deze 19 ZvC-geassocieerde zogenaamde "single-nucleotide" polymorfismen (SNPs) waren beschikbaar van 310 ZvC patiënten en 976 controles uit eerdere studies door onze groep. We bestudeerden de allelische associatie van deze 19 SNPs in ZvC patiënten gestratificeerd voor actief roken bij diagnose en voor passief roken in de kindertijd. Data over actief roken bij diagnose en passief roken in de kindertijd werden verkregen door de eerder genoemde schriftelijke enquête en statusonderzoek. De loci geassocieerd in rokende, maar niet in niet-rokende, ZvC patiënten waren 5p13.1 (rs17234657), *DLG5* (rs2165047), *NKX2-3* (rs10883365) en *NOD2* (R702W). De loci geassocieerd in niet-rokende, maar niet in rokende, ZvC patiënten waren *IL23R* (rs7517847), 5p13.1 (rs9292777), *IRGM* (rs13361189 en rs4958847), *IL12B* (rs6887695) en *CCNY* (rs3936503). *PTPN2* (rs2542151) was alleen geassocieerd in het rokende ZvC cohort (p=0.04), en niet in het gehele ZvC cohort (p=0.23) of in het niet-rokende ZvC cohort (p=0.80). In passief rokende ZvC patiënten werden associaties met 13 SNPs in 9 loci gevonden, inclusief *PTPN2*. In ZvC patiënten niet blootgesteld aan passief roken werden alleen associaties gevonden met *NOD2* (1007fsinsC en G908R). Concluderend vonden we verschillende genetische associaties in verschillende groepen, die zich van elkaar onderscheidden door al dan niet actief roken ten tijde van diagnose en al dan niet passief roken in de kinderleeftijd. Een deel van dit verschil kan verklaard worden door een gebrek aan "power" in dit relatieve kleine ZvC cohort. Toch impliceert het verschil in geassocieerde genen tussen rokende en niet-rokende ZvC patiënten een complexe gen-omgevingsinteractie. Daarom moeten genetische studies in de ZvC gestratificeerd worden voor rookgedrag, want anders kunnen minder sterk geassocieerde genen zoals *PTPN2* gemist worden.

Het is onbekend waarom roken gunstig is voor CU. Eén van de mogelijk betrokken pathways is de heme-oxygenase-1 (HO-1) pathway. HO-1 is het snelheidsbeperkende enzym betrokken bij de afbraak van haem, wat leidt tot de eindproducten biliverdine, Fe^{2+} en koolstofmonoxide. Haem veroorzaakt oxidatieve stress, terwijl alle drie de eindproducten

antioxidatieve, antiapoptotische en anti-inflammatoire eigenschappen hebben. Inductie van HO-1 had een gunstig effect in diermodellen met darmschade. Sigarettenrook (SR) is in staat tot het induceren van HO-1 in verschillende humane cellen, maar het effect van roken op HO-1 expressie in de dikke darm is onbekend. Inductie van HO-1 in de dikke darm zou gunstig kunnen zijn voor CU patiënten door bescherming te bieden tegen de ontwikkeling van CU en/of door ontsteking in geval van actieve CU te verminderen. In hoofdstuk 6 hebben we een laboratoriumonderzoek verricht naar de effecten van roken op de expressie van HO-1 in de dikke darm *in vitro*, in dieren en in mensen. Voor de *in vitro* proeven werden DLD-1 cellen geïncubeerd met sigarettenrook extract (SRE), voor de dierproeven werden muizen blootgesteld aan SR en voor het humane deel werden dikke darmbiopten verzameld van gezonde niet-rokers en rokers. HO-1 expressie werd beoordeeld met quantitative polymerase chain reaction (qPCR), western blotting en immunohistochemie. Cytochroom P450 1A1 (CYP1A1) expressie (qPCR) werd gebruikt als een positieve controle voor blootstelling van de dikke darm aan SR. SRE induceerde dosisafhankelijk HO-1 en CYP1A1 expressie in DLD-1 cellen. In muizen en mensen was de CYP1A1 mRNA expressie in de dikke darm verhoogd bij expositie aan SR, maar HO-1 mRNA en eiwit expressie in de dikke darm was gelijk tussen controles en aan SR geëxposeerden. In de humane studie was de expositie aan SR in de therapeutische bandbreedte voor CU. De conclusie van deze studie is dat SR een CYP1A1 respons induceert, maar geen HO-1 stress respons in epitheel cellen van de dikke darm. Dit wijst erop dat het gunstige effect van SR op UC niet verloopt via opregulatie van HO-1 in de dikke darm, maar SR zou wel andere beschermingsmechanismen lokaal in de dikke darm in gang kunnen zetten.

Literatuur betreffende de rol van roken in ontvangers van een levertransplantatie (LT) is schaars. De paar beschikbare studies over de effecten van roken in LT ontvangers tonen dat roken een risicofactor is voor verschillende complicaties en ernstige aandoeningen na LT. Gezien dit schadelijke effect van roken na LT is het teleurstellend dat slechts twee transplantatiecentra het rookgedrag van LT ontvangers hebben onderzocht. In hoofdstuk 7 beschrijven we het rookgedrag van LT ontvangers voor en na transplantatie, en de associatie tussen roken en het optreden van maligniteiten en cardiovasculaire ziekten na LT. Eén van de doelen was het definiëren van groepen die vatbaar zijn voor herstarten van roken na LT. Alle 401 volwassen patiënten met een follow-up van minimaal twee jaar na LT werden geïncludeerd. Data werden verzameld vanuit medische statussen en naar alle 326 levende patiënten werd een enquête gestuurd over het rookgedrag op vier momenten voor en na LT. Op de enquête reageerden 301 (92%) patiënten. Zowel voor als na LT gebruikte 53% van de patiënten nooit tabak en ongeveer 17% was actief roker. Ter vergelijk, in 2007 rookte 28% van de Nederlandse bevolking. Bijna een derde van de actieve rokers tijdens evaluatie voor LT lukte het om te stoppen met roken, meestal tijdens de wachtlijstperiode voor LT. Twaalf procent van de ex-rokers begon opnieuw met roken, vooral na LT. Vooral ex-rokers die nog maar een relatief korte tijd geleden gestopt waren, waren vatbaar voor een terugval. Tabakgebruik was het hoogst in patiënten met een alcoholische leverziekte (52% was actief roker voor LT en 44% na LT) en het laagst in patiënten met primaire scleroserende cholangitis (1,4% was actief roker voor LT). Tien jaar na LT was het cumulatieve aantal maligniteiten 12,7% in actieve rokers tegen 2,1% in niet-rokers (P=0.02). Er werd geen associatie gevonden met huidkanker of cardiovasculaire ziekten. Concluderend, voor en na LT zijn ongeveer 17% van de patiënten actief roker. Na LT omvat dit percentage ex-rokers

die weer zijn begonnen en een afname van actieve rokers bij patiënten met een alcoholische leverziekte. Preventieprogramma's moeten zich niet alleen focussen op actieve rokers, maar ook op ex-rokers en dan vooral ex-rokers die recent zijn gestopt. Gezien het hoge aantal maligniteiten in actieve rokers na LT zijn een interventieprogramma na LT en frequente screening voor maligniteiten nodig.

Toekomstperspectieven

Ondanks de enorme hoeveelheid studies over de effecten van roken op het ziektebeloop van de ZvC en CU is het nog steeds onduidelijk welke rol roken precies speelt in het beloop van beide ziekten. In dit proefschrift konden wij geen nadelig effect van roken vinden op het beloop van de ZvC, terwijl andere studies wel een duidelijk nadelig effect vonden. Waarschijnlijk is roken alleen nadelig voor bepaalde patiënten met de ZvC. Dit zou afhankelijk kunnen zijn van geslacht, en lokalisatie en ernst van de ziekte. Eerder is al gesuggereerd dat roken vooral voor vrouwelijke ZvC patiënten nadelig is, maar dit konden wij in dit proefschrift niet bevestigen. Toekomstige studies zouden meer moeten focussen op het identificeren van specifieke patiëntgroepen die vatbaar zijn voor de effecten van roken. Wij hebben als eerste de effecten van passief roken op het beloop van IBD onderzocht, en hebben dit gedaan in zowel een cohort van een academisch als van een regionaal ziekenhuis. In het academische cohort vonden we een nadelig effect van passief roken bij ZvC patiënten, maar konden dit niet bevestigen in het regionale cohort. Er zijn dan ook meer studies over de effecten van passief roken op het beloop van IBD nodig om de precieze rol van passief roken te bepalen.

In dit proefschrift hebben we laten zien dat de ontwikkeling van de ZvC mede afhankelijk is van een interactie tussen roken en de genetische aanleg. Ook de effecten van rook op het ziektebeloop zouden weleens afhankelijk kunnen zijn van de genetische aanleg. Hopelijk lukt het in de toekomst genetische varianten te selecteren die patiënten vatbaar maken voor de effecten van roken. Op dit moment is het nog niet mogelijk het beloop van de ZvC te voorspellen, maar er gloort hoop. Onderzoekers van onze groep toonden aan dat het misschien mogelijk wordt subgroepen van ZvC patiënten met een ernstige prognose te identificeren op basis van de genetische varianten. Zij vonden een associatie tussen een toenemend aantal risicoallelen en een ernstiger ziektebeloop.[1] Komende studies moeten zich richten op het classificeren van ZvC patiënten gebaseerd op genetische aanleg in combinatie met rookgedrag. Hopelijk kunnen we hierdoor specifieke patiëntgroepen identificeren en hierop de behandeling aanpassen; sommige patiënten kunnen baat hebben bij vroege interventie en agressieve medicatie, terwijl andere patiënten juist de bijwerkingen van onnodige medicatie bespaard kan blijven.

Uit onze studie naar de interactie tussen roken en genetische aanleg blijkt duidelijk dat specifieke omgevingsfactoren nodig zijn om bepaalde genetische varianten te laten bijdragen aan het ontwikkelen van ziekte. Dit is een belangrijke bevinding, omdat de meeste genetische patiënt-controle studies niet gestratificeerd zijn voor omgevingsfactoren en niet gecorreleerd zijn aan omgevingsfactoren in de controle cohorten. Als deze studies wel gaan stratificeren voor rookgedrag en/of andere omgevingsfactoren is het misschien mogelijk om nog meer ZvC-geassocieerde genen te vinden en waarschijnlijk ook meer CU-geassocieerde genen. Dit zal vooral het geval zijn voor geassocieerde genen met lage odds ratio's.

Hoewel de effecten van roken op het ontstaan en beloop van IBD al decennia worden bestudeerd, is het nog steeds onbekend welke pathways beïnvloed worden door roken en welke component(en) in sigarettenrook verantwoordelijk is/zijn. In dit proefschrift hebben we de heme-oxygenase 1 (HO-1) pathway bestudeerd. Hoewel sigarettenrook HO-1 expressie induceerde in *in vitro* experimenten met DLD-1 cellen, vonden we geen effect van roken op de expressie van HO-1 in de dikke darm van muizen en mensen. Dit betekent echter niet dat de HO-1 pathway niet betrokken is bij de gunstige effecten van roken op CU, want roken zou zijn gunstige effecten ook kunnen uitoefenen door opregulatie van HO-1 in de longen met als gevolg verhoogde koolstofmonoxide concentraties. Verschillende interventiestudies in muizen hebben gunstige effecten van koolstofmonoxide aangetoond bij darmschade.[2-4] Wellicht blijkt uit toekomstige interventiestudies dat koolstofmonoxide ook gunstig is voor CU patiënten. In dit proefschrift hebben we aangetoond dat sigarettenrook de dikke darm bereikt gezien de verhoogde CYP1A1 expressie in de dikke darm van rokers. Dit betekent dat sigarettenrook andere lokale beschermingsmechanismen dan HO-1 in de dikke darm kan beïnvloeden. Om deze te vinden, zijn meer studies nodig.

Gezien de mogelijk nadelige effecten van roken op de ZvC moet stoppen met roken één van de therapeutische doelen zijn. In dit proefschrift toonden we aan dat de helft van de bij diagnose rokende ZvC patiënten stopte met roken na de diagnose en we concludeerden dat ZvC patiënten vatbaarder zijn voor het stoppen met roken dan de algemene populatie. Een Franse studie vond dat na interventie het slechts 12% van de rokers lukte om meer dan een jaar te stoppen en slechts 10% lukte het om langdurig te stoppen.[5] Een recente studie suggereerde dat de meeste ZvC patiënten succesvol in de IBD kliniek geholpen kunnen worden bij het stoppen met roken zonder specialistische hulp, aangezien ZvC patiënten een hoog kennisniveau over de risico's van roken op de gezondheid hadden en een lage nicotineafhankelijkheid.[6] In tegenstelling tot deze studie toonde een andere studie aan dat ZvC patiënten vaker het rookgerelateerde persoonlijkheidskenmerk "impulsive sensation seeking" hadden, wat juist geassocieerd is met een geringe neiging tot stoppen.[7] De auteurs van deze studie suggereren dat ZvC patiënten met "impulsive sensation seeking" specifieke strategieën voor het stoppen met roken nodig hebben. Bovenstaande studies zijn dus niet eenduidig en er zijn meer studies nodig om opheldering te krijgen of ZvC patiënten speciale stopprogramma's nodig hebben en hoe deze er dan uit moeten zien.

Elke maag-darm-leverarts is het er mee eens dat het advies aan ZvC patiënten is om te stoppen met roken. De situatie voor CU is gecompliceerder. Zolang we niet weten welke component in sigarettenrook de gunstige effecten veroorzaakt, blijft het moeilijk wat we CU patiënten moeten adviseren. Het is wel belangrijk dat we CU patiënten die willen stoppen met roken niet ontmoedigen, want op de lange termijn worden ze beschermd tegen rookgerelateerde ziekten; CU patiënten hadden een verminderd risico op longkanker [8,9] en hart- en vaatziekten [8,10] vergeleken met ZvC patiënten.

De tweede focus van dit proefschrift is de rol van roken in LT ontvangers. Voor patiënten die geëvalueerd worden voor LT is stoppen met alcohol een voorwaarde om in aanmerking te komen voor LT, vooral voor diegenen met een alcoholische leverziekte. De vraag is of stoppen met roken ook ingevoerd moet worden als een voorwaarde om in aanmerking te komen voor LT. In dit proefschrift vonden wij dat voor en na LT ongeveer 17% van de patiënten actief roken en dat actieve rokers na LT zes keer vaker maligniteiten hebben. Andere studies hebben ook ongunstige effecten van roken op de lange termijn na LT aangetoond en dus moet (blijvend) stoppen met roken een belangrijk doel na LT zijn. Of stoppen met roken

ook een belangrijk doel vóór LT moet zijn, zal moeten blijken uit toekomstige studies over de effecten van roken op acute complicaties na LT, zoals trombose van de leverslagader [11] en transplantaatfalen. Interessant is een recente studie waarin actieve rokers een 92% hoger aantal galwegcomplicaties hadden na LT dan nooit-rokers.[12] Mede gezien deze laatste studie en het opmerkelijk lage aantal van 1,4% actieve rokers bij primaire scleroserende cholangitis zoals aangetoond in dit proefschrift, zou het interessant zijn om te bestuderen wat het effect van roken is op recidief van primaire scleroserende cholangitis na LT. Dit zal echter alleen mogelijk zijn in een multicenter studie gezien het lage aantal rokers in deze patiënt groep.

Concluderend kan worden gesteld dat studies, inclusief die in dit proefschrift, ons hebben voorzien van enorm veel kennis over de effecten van roken op de ZvC en CU. De ware rol van roken bij ZvC en CU is echter nog steeds niet duidelijk en nog belangrijker, we weten nog steeds niet welke mechanismen door roken worden beïnvloed. Het laatste decennium is een nog steeds groeiend aantal genetische varianten gevonden die geassocieerd zijn met IBD. In dit proefschrift hebben we een interactie aangetoond tussen deze genetische varianten en rookgedrag op de ontwikkeling van de ZvC. Deze bevindingen bewijzen dat toekomstige genetische studies in de ZvC en CU gestratificeerd moeten worden voor rookgedrag. In LT ontvangers zijn hart- en vaatziekten en maligniteiten belangrijke oorzaken van morbiditeit en mortaliteit. In dit proefschrift toonden we een hoog en constant aantal rokers voor en na LT, en een associatie tussen roken en maligniteiten na LT. Dit maakt dat een interventieprogramma voor het stoppen met roken en frequente screening op maligniteiten bij rokers nodig zijn in LT ontvangers.

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List of publications

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Curriculum Vitae

Curriculum Vitae

Frans van der Heide is geboren op 1 oktober 1981 te Ternaard, Friesland. In 2000 behaalde hij zijn VWO-diploma aan het Dockinga College te Dokkum. September 2000 startte hij met de studie Geneeskunde aan de Rijksuniversiteit Groningen. De co-schappen werden gedaan in het Universitair Medisch Centrum Groningen (UMCG) en een aantal affiliatie ziekenhuizen van het UMCG. Het keuze co-schap werd gedaan op de afdeling interne geneeskunde/maag-, darm- en leverziekten (MDL) in het Medisch Centrum Leeuwarden. In januari 2007 behaalde hij cum laude het artsexamen. Februari 2007 begon hij als AGNIO bij de hepatologie en levertransplantatie van de afdeling MDL in het UMCG. Naast de werkzaamheden als AGNIO, werd er ook onderzoek gedaan naar de invloed van roken bij patiënten met chronische inflammatoire darmziekten en bij mensen na levertransplantatie. Sinds 1 augustus is hij begonnen met de vooropleiding interne geneeskunde in het Medisch Spectrum Twente te Enschede (opleider Dr. W.M. Smit). Vanaf 1 augustus 2011 wordt de vervolgopleiding tot MDL-arts voortgezet in Enschede (opleider Dr. J.J. Kolkman) en vanaf 1 augustus 2013 in het UMCG (opleider Prof. Dr. J.H. Kleibeuker).

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Dankwoord

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